VOLUME 66 • AUGUST 1958 • NUMBER 2

PATHOLOGY

A Periodical Devoted to General and Experimental Pathology

AMERICAN MEDICAL ASSOCIATION PUBLICATION

TABLE OF CONTENTS

VOLUME 66 AUGUST 1958 NUMBER 2

ORIGINAL ARTICLES

OBITUARIES

REGULAR DEPARTMENTS

A. M. A.
ARCHIVES of PATHOLOGY

Also the Official Organ of the AMERICAN SOCIETY FOR EXPERIMENTAL PATHOLOGY

VOLUME 66 AUGUST 1958 NUMBER 2

SUBSCRIPTION RATES

Price per annum in advance, including postage: Domestic, $10.00. Canadian, $10.50. Foreign, $11.50. Price to students, interns, and residents, $6.00 in U. S. & possessions.

Single copies of this and previous calendar year, $1.00 each. Back issues older than two years are available through Walter J. Johnson, Inc., 111 Fifth Avenue, New York 3. Future reprints of back issues will be available through Johnson Reprint Corporation, 111 Fifth Avenue, New York 3.

Checks, money orders, and drafts should be made payable to the American Medical Association, 535 North Dearborn Street, Chicago 10.

AMERICAN MEDICAL ASSOCIATION Publication

Published monthly by the AMERICAN MEDICAL ASSOCIATION. Editorial and Circulation Offices: 535 North Dearborn Street, Chicago 10, Illinois. Publication Office: Thompson Lane, Box 539, Nashville 1, Tennessee. Change of Address: Notice to the circulation office should state whether or not change is permanent and should include the old address. Six weeks' notice is required to effect a change of address. Second-class mail privileges authorized at Nashville, Tenn., Aug. 6, 1956.

Paragon Tray Drawer Cabinet

Compact

U. S. Pat. No. 2,202,047
C101—Tray Drawer Cabinet for 3 x 1 Micro Slides
Capacity 4500—18¾ x 15¾ x 4¾

Low Cost

FOR FILING
MICROSCOPIC SLIDES 3 x 1"
KODACHROME TRANSPARENCIES
2 x 2" SLIDES
LANTERN SLIDES
(up to 3¼ x 4¼)
PETROGRAPHIC SLIDES

When you purchase a
PARAGON TRAY DRAWER CABINET
YOU PURCHASE FILING SPACE ONLY
NO WASTE SPACE-EVERY INCH USED

All Paragon Tray Drawer Cabinets are manufactured in standard sizes so that any number of sections may be interlocked to form one cabinet to accommodate any number of varied slides. The dimensions of the different cabinets are the same as to length and width, varying only in height. The cabinet formed by interlocking may be 18¾ x 15¾; 18¾ x 11 or 18¾ x 5 or it may be a pyramid with the sections varying in width.

C221—Capacity 1500 Slides—18¾ x 11 x 3⅞
For Filing KODACHROME TRANSPARENCIES and 2 x 2" SLIDES

SPECIFICATIONS: All Paragon Tray Drawer Cabinets are made of reinforced steel construction, olive green finish. Interlocking device enables several units to be joined into one. Each sectional unit contains removable drawers with hand grip in front and rear. Interlocking steel base obtainable whenever required. **Constructed according to rigid specifications—not merely adapted.**

Address your orders and inquiries to Dept. P.

Manufactured Exclusively by

PARAGON C. & C. CO., Inc. • 2540 Belmont Ave., New York 58, N.Y.

Instructions to Contributors

Articles, book reviews, and other materials for publication should be addressed to the Chief Editor. Articles are accepted for publication on condition that they are contributed solely to this journal.

An original typescript of an article, with one carbon copy, should be provided; it must be double or triple spaced on one side of a standard size page, with at least a 1-inch margin at each edge. Another carbon copy should be retained by the author.

The main title of an article may not contain more than eighty characters and spaces; a subtitle may be of any length.

The author's name should be accompanied by the highest earned academic or medical degree which he holds. If academic connections are given for one author of an article, such connections must be given for all other authors of the article who have such connections.

If it is necessary to publish a recognizable photograph of a person, the author should notify the publisher that permission to publish has been obtained from the subject himself if an adult, or from the parents or guardian if a child. An illustration that has been published in another publication should be accompanied by a statement that permission for reproduction has been obtained from the author and the original publisher.

Oversized original illustrations should be photographed and a print on glossy paper submitted. Prints of a bluish tinge should be avoided. Large photomicrograph prints will be reduced in scale unless portions to be cropped are indicated by the author. The author should submit duplicate prints of roentgenograms and photomicrographs with the essential parts that are to be emphasized circled, as a guide to the photoengraver.

Charts and drawings should be in black ink on hard, white paper. Lettering should be large enough, uniform, and sharp enough to permit necessary reduction. Glossy prints of x-rays are requested. Paper clips should not be used on prints, since their mark shows in reproduction, as does writing on the back of prints with hard lead pencil or stiff pen. Labels should be prepared and pasted to the back of each illustration showing its number, the author's name, and an abbreviated title of the article, and plainly indicating the top. Charts and illustrations must have descriptive legends, grouped on a separate sheet. Tables must have captions. ILLUSTRATIONS SHOULD BE UNMOUNTED.

References to the literature should be limited to those used by the author in preparation of the article. They should be typed on a special page at the end of the manuscript. The citation should include, in the order given, name of author, title of article (with subtitle), name of periodical, with volume, page, month—day of month if weekly or biweekly—and year. References to books must contain, in the order given, name of author, title of book, city of publication, name of publisher, and year of publication.

AMERICAN MEDICAL ASSOCIATION

535 North Dearborn Street **Chicago 10**

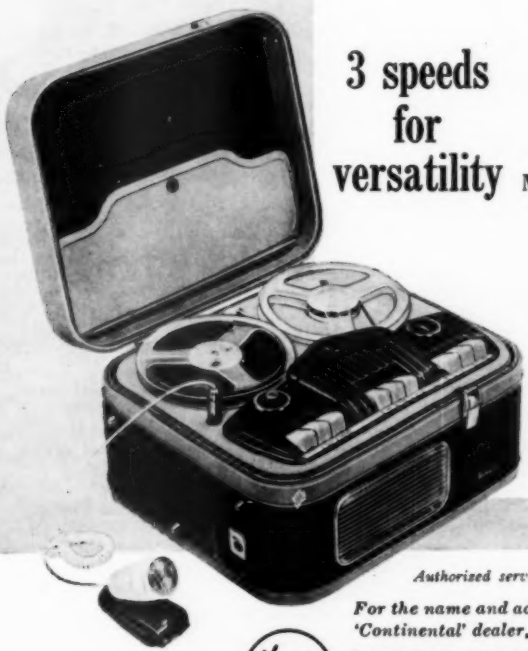
HE uses the 'Continental' at its SLOW speed

HE uses the 'Continental' at its MEDIUM speed

THEY use the 'Continental' at its FAST speed

the all-in-one *portable* tape recorder engineered by Philips of the Netherlands

NORELCO® 'Continental'



3 speeds for versatility

SLOW $1\frac{7}{8}$ inches per second
designed for *speech* – with the ultimate in tape economy

MEDIUM $3\frac{3}{4}$ inches per second
the perfect "compromise" speed—for critical speech recording as well as music

FAST $7\frac{1}{2}$ inches per second
for genuine high-fidelity music reproduction

Top-quality dynamic microphone included with each unit.

Authorized service and maintenance facilities in all major cities.

For the name and address of your nearest 'Continental' dealer, write to:

NORTH AMERICAN PHILIPS CO., INC.
High Fidelity Products Division, Dept.–IT 8
230 DUFFY AVENUE, HICKSVILLE, L. I., N. Y.

The NORELCO 'Continental' is available in Canada as the "Philips TR3."

ALCOHOLISM

an important problem in today's living!

The following articles from TODAY'S HEALTH are now available in one pamphlet for 50 cents . . .

ALCOHOLICS ANONYMOUS. Written from the standpoint of a member, the basic treatment procedures are described and the psychological problems confronting the alcoholic are discussed.

ALCOHOL AND CIRRHOSIS OF THE LIVER. Relationship between alcohol, diet and cirrhosis. Increasing stress on nutritional differences. by Russell S. Boles

HOW TO HELP A PROBLEM DRINKER. Understanding the alcoholic's capabilities, the necessity of help, causes of his condition. by Edward A. Strecker and Francis T. Chambers, Jr.

THE TREATMENT OF ALCOHOLISM. Tracing the steps from convincing the alcoholic that he is sick through treatment and cure. by Lewis Inman Sharp

CONDITIONED REFLEX TREATMENT OF CHRONIC ALCOHOLISM. Its place among methods of treatment today, its development and correlation with personality factors. by Walter L. Voegtlin

INSTITUTIONAL FACILITIES FOR THE TREATMENT OF ALCOHOLISM. Comparative differences, in drinking with the last century, new establishments and methods of treatment, lack of trained personnel. by E. H. L. Corwin

other pamphlets available . . .

ALCOHOLISM IS A DISEASE. A discussion by the Chairman of the A.M.A.'s Committee on Alcoholism. by Marvin A. Block, M.D., 8 pages, 15 cents

I AM THE WIDOW OF AN ALCOHOLIC. Three articles combined by Virginia Conroy, 16 pages, 20 cents

HOW EXPERTS MEASURE DRUNKENNESS. A partial transcript of an actual courtroom case. by H. A. Heise, 8 pages, 15 cents

BARBITURATES, BOOZE AND OBITUARIES. A discussion of the dangers of mixing alcohol and barbiturates. by Donald A. Dukelow, 4 pages, 10 cents

TWELVE STEPS FOR ALCOHOLICS. A frank discussion of the meaning of an alcoholic behavior. by Richard Lake, 6 pages, 10 cents

address requests to . . .

ORDER DEPARTMENT

AMERICAN MEDICAL ASSOCIATION
535 NORTH DEARBORN STREET, CHICAGO 10, ILLINOIS

A.M.A. ARCHIVES OF
PATHOLOGY

Chemical Pathology of Acute Amino Acid Deficiencies

I. Morphologic Changes in Immature Rats Fed Threonine-, Methionine-, or Histidine-Devoid Diets

HERSCHEL SIDRANSKY, M.D., and EMMANUEL FARBER, M.D., Ph.D., New Orleans

There is increasing evidence that both the quantity and composition of dietary protein are important factors in the pathogenesis of certain forms of fatty liver. In animal experiments, low-protein diets adequate in choline,[1-4] diets with protein of poor quality,[5] diets with amino acid imbalances,[6] and diets devoid of single essential amino acids[7-12] have all been reported to induce a fatty liver. Severe fatty liver is one of the most constant pathologic findings in kwashiorkor, a systemic disorder which is geographically widespread. It is generally accepted that an inadequate amount of high-quality protein in the diet is the most important factor in the pathogenesis of kwashiorkor.[13] In most forms of fatty liver induced experimentally by protein deficiency, as well as in kwashiorkor, the excess liver lipid shows a characteristic periportal distribution,[1-5,7,8,10,14] in contrast to the fatty liver in animals given choline-devoid methionine-poor diets in which the fat first appears about central veins.[15]

Submitted for publication March 20, 1958.

Departments of Pathology and Biochemistry, Tulane University School of Medicine. Life Insurance Medical Research Fellow 1956-1957 (Dr. Sidransky).

Supported in part by research grants from the National Institute of Arthritis and Metabolic Diseases, United States Public Health Service (A-610), and the Life Insurance Medical Research Fund.

Although the relationship between fatty liver and dietary deficiencies of protein or amino acids has been established, there is as yet little information about its mechanism. Some studies of enzyme activities in the livers of children with periportal fatty change have been reported,[16,17] but the findings have not led to any clear concept of the pathogenesis of the condition.

Deficiencies of dietary protein or essential amino acids interfere with the normal processes of protein synthesis. It is therefore possible that inhibition of hepatic protein synthesis might be concerned in the pathogenesis of the fatty livers that develop in protein-deficiency states.[18] Evidence in support of this concept was obtained from studies on the morphologic and biochemical alterations in the livers of rats given injections of ethionine, a metabolic antagonist of methionine. In these animals definite inhibition of hepatic protein synthesis was demonstrated and was believed to bear a relationship to the subsequent development of a periportal fatty liver.[19]

One of the purposes of the present investigation, therefore, was to determine whether a correlation between disturbed hepatic protein metabolism and periportal fatty liver could also be demonstrated when the liver changes were induced by dietary deficiencies of amino acids. The force-feeding of diets devoid of certain single essential amino acids was used to

produce the fatty liver, and some aspects of hepatic protein metabolism were studied in these animals.

Using a force-feeding technique with tryptophan-, isoleucine-, or phenylalanine-devoid diets, Adamstone and Spector [7] and Samuels and co-workers [8-11] observed the rapid development of periportal fatty liver in their animals. These results contrasted sharply with those of other investigators, who reported no fatty liver when animals were fed ad libitum similar diets or diets deficient in other essential amino acids.[20-27] The different results in these two types of studies is most probably due to the undernutrition which accompanies the ad libitum-feeding but not the force-feeding of the deficient diets. For our study, therefore, we selected a force-feeding regimen in order to avoid the complication of severe undernutrition.

An additional purpose of this investigation was to observe whether pathologic changes in organs other than the liver, similar to those seen in kwashiorkor, could be induced by force-feeding diets devoid of a single essential amino acid and to study some of the biochemical changes accompanying the morphologic alterations. A preliminary report by Samuels and co-workers [8] indicated that pancreatic atrophy and other lesions simulating the changes seen in kwashiorkor could be induced by force-feeding rats diets devoid in tryptophan, isoleucine, or phenylalanine.

In the present paper, the gross and microscopic alterations seen in rats within the first week of the force-feeding of diets devoid in each of three amino acids—threonine, methionine, or histidine—are presented. The subsequent paper presents the biochemical changes observed within the same period in the animals fed the threonine- or methionine-devoid diets. In addition, morphologic and biochemical data in animals fed identical diets ad libitum are presented in this and the subsequent paper and compared with the changes found in the force-feeding experiments.

Experimental Data

Male and female Sprague-Dawley rats, one month old and weighing on the average 80 gm., were used. The animals were maintained with Purina Laboratory Chow for one week and then fasted overnight before the experiments were begun. In all experiments, several groups of rats, each of the same sex, age, and weight, were used. Rats were housed in individual wire cages in an air-conditioned room.

The basal experimental diet was based upon that used by Forbes and Vaughan [12] and was composed of the following constituents: essential amino acids 9.2%, nonessential amino acids 8.1%, salt mixture 4%, vitamin sucrose mixture 5%, corn oil 5%, cod liver oil 1.5%, and sucrose 67.2%. Essential amino acids were provided in the following quantities: L-lysine HCl 1.24%, L-arginine HCl 0.75%, DL-tryptophan 0.20%, DL-phenylalanine 0.90%, DL-leucine 1.60%, DL-isoleucine 1.00%, DL-threonine 1.00%, DL-valine 1.40%, DL-methionine 0.60%, and L-histidine HCl 0.54%. The nonessential amino acids consisted of L-glutamic acid 2.00%, DL-serine 0.50%, glycine 0.70%, L-tyrosine 1.40%, L-cystine 0.20%, L-proline 0.90%, L-asparagine 1.22%, and DL-alanine 1.20%. The vitamin-sucrose mixture contributed the following number of milligrams of vitamins to each 100 gm. of diet: thiamine HCl 0.25, riboflavin 0.5, pyridoxine HCl 0.25, calcium pantothenate 2.0, nicotinamide 1.0, choline chloride 100.0, biotin 0.01, folic acid 0.1, inositol 10.0, 2 methyl-1,4,-naphthoquinone 0.1, and cyanocobalamin (vitamin B_{12}) 0.01. The salt mixture was that described by Hegsted et al.[28]

The ration was blended with water so that each milliliter of diet mixture contained 1 gm. of diet. This yielded a thick slurry suitable for administration by stomach tube. Each group of experimental animals received rations devoid of only a single essential amino acid, either threonine, methionine, or histidine. Sucrose was substituted for the missing amino acid. Control rats received the complete ration. In some experiments the rats of all groups received from one-half to two days of force-feeding of the complete diet before beginning the experimental diets.

Force-feeding was performed with plastic tubes by the method of Shay and Gruenstein.[29] The diet was given twice daily, at 8:30 a. m. and 4:30 p. m. The rats received an average daily feeding of 0.9 gm. of ration per 10 gm. of initial body weight. Rats receiving ad libitum feedings had diet available at all times. All animals had free access to water.

Groups of rats were killed after three, six, or seven days and were killed in the morning, approximately 18 hours after their last feeding. The animals were anesthetized with ether and killed by

exsanguination. The findings were identical in animals given the diets for six and for seven days. They are therefore treated in the results as one group.

Rats were weighed at the beginning of each experiment and again at the time of killing. The liver and pancreas were weighed fresh, and their dry weights were then determined by heating a weighed aliquot at 103 C for 48 hours. Pieces of tissue from various organs were fixed in Bouin's solution and in 10% formalin. Paraffin sections were prepared and stained with hematoxylin and eosin. Some were stained with Best's carmine and periodic acid-Schiff stains. Frozen sections of liver were stained with Sudan IV.

Results

Appearance of the Animals.—In the force-feeding experiments, the control rats were normal in general appearance and were indistinguishable from rats fed the stock diet. In contrast, the hair of the animals of the experimental groups became rough and shaggy within a few days after the beginning of the deficient diets. This became progressively worse, and in some rats, areas of alopecia developed on the head and hindlegs. After four to five days of the diets, the experimental rats developed a dull reddish pigmentation around the mouth. They also became weak and lethargic. Some rats were hyperreactive to stimuli such as noise or handling. The experimental rats struggled to resist the force-feeding, in contrast to the control animals, which submitted quietly to the feeding procedure. These changes were more obvious in the threonine- and histidine-devoid than in the methionine-devoid groups. Frequently, rats in the threonine-devoid group died before seven days. Neither bloating nor diarrhea was observed in any of the animals that were force-fed control or devoid diets. In the ad libitum experiments, rats given the deficient diets weighed less than the controls at time of killing but no other gross differences were observed.

Changes in Body and Organ Weights.—Table 1 summarizes the changes in the weights of the whole body, the liver, and the pancreas in the control and experimental rats force-fed for six and seven days. Even though the amount of food administered to all animals was the same in each experiment, the control rats gained while the experimental rats lost weight.

The mean wet weight of the liver in animals given the threonine-devoid but not those given the methionine- or histidine-devoid diets was significantly elevated above that of the controls. The per cent dry weight, however, was the same in all livers. The mean wet weight of the pancreas of the animals in the threonine-devoid group was significantly greater than that in the control group, but the dry weight of the pancreas was 3.5% less.

The dietary consumption, organ weights, and relative body weights of animals fed ad libitum for seven days (Table 2) were strikingly different than those in the force-fed groups. While the force-fed rats received the same amount of ration in each group, the animals in the groups fed ad libitum consumed different amounts, depending upon the composition of the diet.

TABLE 1.—*Changes in Body, Liver, and Pancreas Weights of Rats Force-Fed Threonine-, Methionine-, or Histidine-Devoid Diets for Seven Days* *

Group	Change in Body Weight, Gm.	Liver		Pancreas	
		Wet Weight, Gm.	% Dry Weight	Wet Weight, Mg.	% Dry Weight
Control	(30) +7.9	(30) 3.10±0.05 †	(8) 29.9±0.9 †	(14) 324±18 †	(2) 23.7±1.0 †
Threonine-devoid	(32) −1.7	(32) 4.21±0.01 ‡	(9) 29.1±4.5 §	(16) 444±15 ‡	(4) 20.2±0.9 §
Control	(23) +8.0	(26) 3.33±0.09	(3) 29.3±0.1	(9) 387±26	--
Methionine-devoid	(22) −6.0	(25) 3.26±0.09 §	(3) 29.7±1.7 §	(9) 383±8 §	--
Control	(9) +8.0	(9) 2.93±0.07	--	--	--
Histidine-devoid	(9) −4	(9) 3.29±0.17 §	--	--	--

* Number in parentheses indicates the number of animals in the group.
† Mean value ± standard error of the mean.
‡ $P<0.01$ (highly significant).
§ $P>0.05$ (not significant).

TABLE 2.—*Diet Consumption and Changes in Body, Liver, and Pancreas Weights of Rats Fed Threonine-, Methionine-, or Histidine-Devoid or Control Diets ad Libitum for Seven Days* *

Group	Diet Consumed, Gm./Rat/7 Days	Change in Body Weight, Gm.	Liver Weight, Gm.	Pancreas Weight, Mg.
Control	62.3	(10) +19	(8) 4.06±0.18 †	(8) 271±37 †
Threonine-devoid	28.7	(10) −10	(8) 2.74±0.08 ‡	(8) 377±11 §
Methionine-devoid	43.4	(10) −9	(8) 2.82±0.11 ‡	(8) 341±14 ‖
Histidine-devoid	32.4	(5) −5	(3) 2.79±0.07 §	(3) 344±25 ‖

* Number in parentheses indicates the number of animals in the group.
† Mean value ± standard error of the mean.
‡ $P<0.01$ (highly significant).
§ P between 0.05 and 0.01 (probably significant).
‖ $P>0.05$ (not significant).

Rats in the control groups ate the largest amount of food and gained weight, while those in the experimental groups ate much less food and lost weight. Also, the livers were heavier in the control animals than in the animals of all of the experimental groups. The weight of the pancreas was increased in the animals given the threonine-, methionine-, and histidine-devoid diets.

Autopsy Findings in Seven-Day ad Libitum Experiment.—The animals fed the devoid diets ad libitum for seven days revealed no specific gross or microscopic alterations on comparison with the controls. This is in sharp contrast to the findings in the force-fed groups.

Autopsy Findings in Seven-Day Force-Feeding Experiments.—Liver: The livers of the control animals contained little fat or glycogen when examined with hematoxylin and eosin and with special stains (Figs. 1, 3, and 5). The majority of nuclei of the hepatic cells contained several small nucleoli (Fig. 7).

In the threonine-devoid rats, the liver was large and had a glistening yellowish-brown appearance. Histologically the hepatic cells were ovoid and greatly distended. The cytoplasm was so diffusely vacuolated as to make it difficult to decide in hematoxylin and eosin preparations whether the infiltrate was primarily glycogen or lipid. However, with special stains, the liver cells

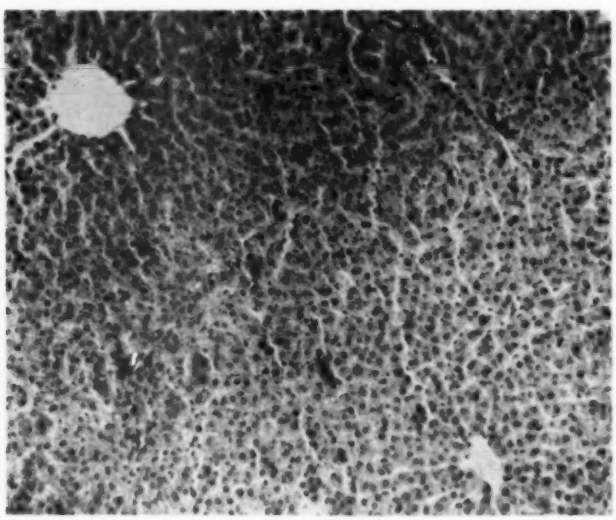


Fig. 1.—Liver of control rat. Hematoxylin and eosin; × 125.

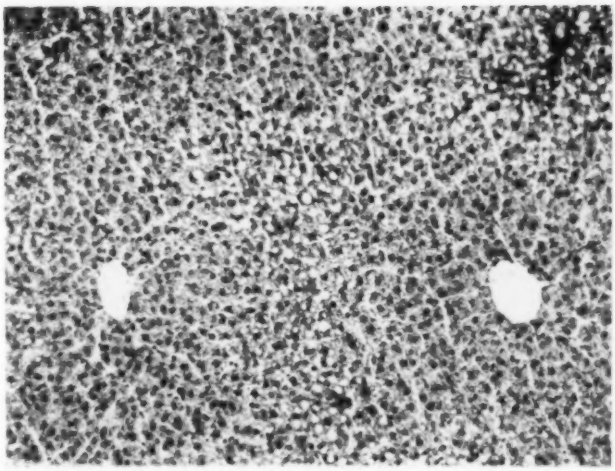


Fig. 2.—Liver of rat force-fed the methionine-devoid diet. Lipid vacuolation is prominent in hepatic cells in periportal areas, while only a few vacuoles are present in central areas. Compare with Figure 1. Hematoxylin and eosin; × 125.

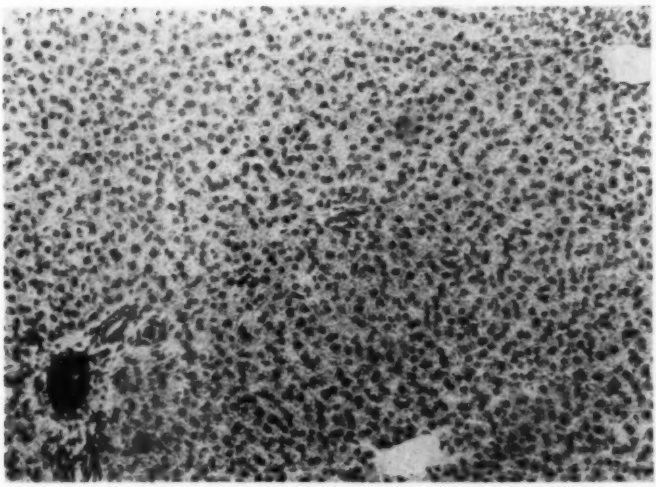


Fig. 3.—Liver of control rat. Sudan IV stain; reduced slightly from mag. × 125.

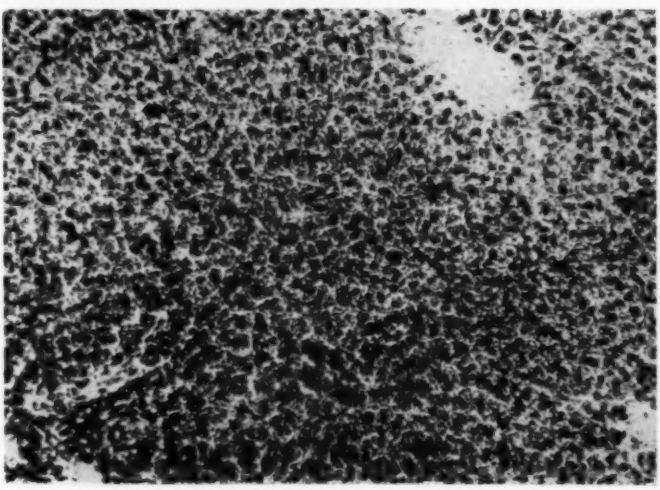


Fig. 4.—Liver of rat force-fed the threonine-devoid diet. Lipid is prominent in hepatic cells in periportal area (lower left). Little lipid is present in cells about the central area. Compare with Figure 3. Sudan IV stain; reduced slightly from mag. × 125.

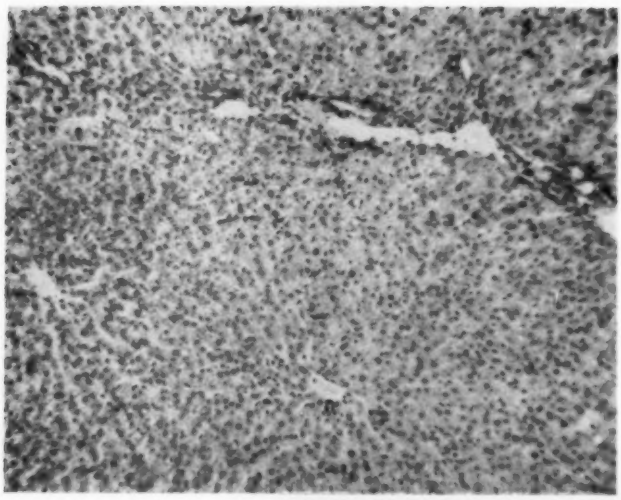


Fig. 5.—Liver of control rat. Best's carmine stain; × 125.

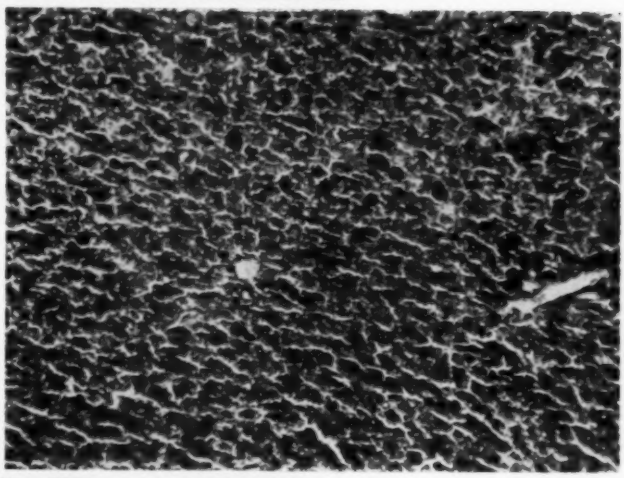


Fig. 6.—Liver of rat force-fed the threonine-devoid diet. A large amount of glycogen is present in all hepatic cells. Compare with Figure 5. Best's carmine stain; × 125.

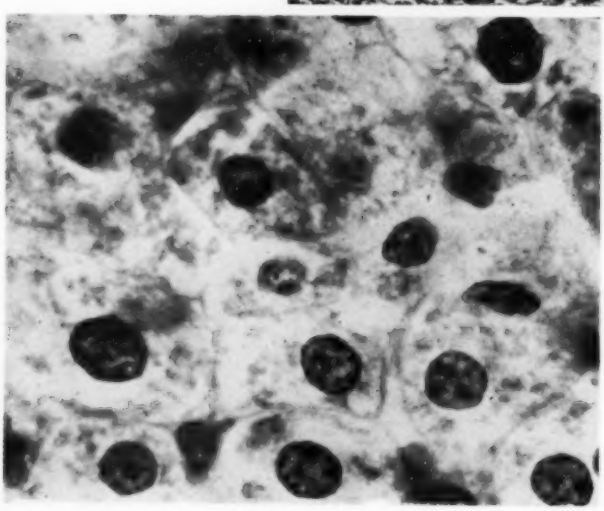


Fig. 7.—Liver of control rat. Oil-immersion view of hepatic cells, showing nuclei containing many small nucleoli. Hematoxylin and eosin; × 1300.

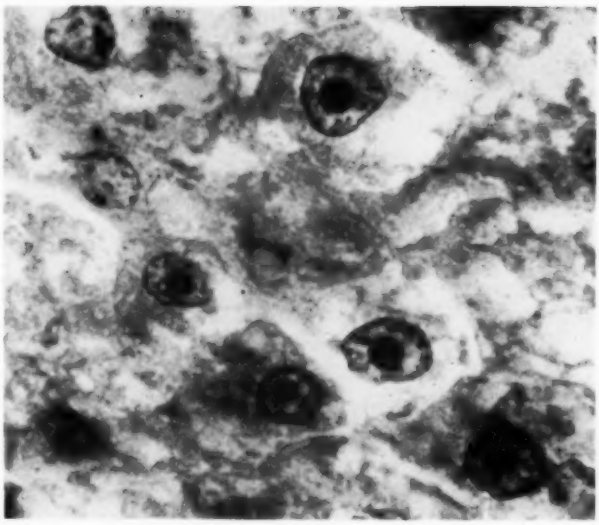


Fig. 8.—Liver of rat force-fed the threonine-devoid diet. Oil-immersion view of hepatic cells, showing nuclei containing single large nucleolus. Hematoxylin and eosin; × 1300.

showed considerable amounts of both glycogen and lipid (Figs. 4 and 6). The lipid was confined to the hepatic cells about the portal triads, whereas the glycogen showed no selective lobular distribution. The hepatic-cell nuclei in most cases contained one large nucleolus instead of several small ones (Fig. 8).

In the methionine-devoid rats, the hepatic cells contained many discrete sudanophilic vacuoles, the larger ones being adjacent to the portal triads and the smaller ones, closer to the central veins (Fig. 2). Best's carmine stain revealed little glycogen. The nuclei and nucleoli were similar to those in the controls.

The livers of the animals given the histidine-devoid diet were similar to those given the threonine-devoid diet except that most of the hepatic-cell nuclei contained a few small nucleoli, as in the controls.

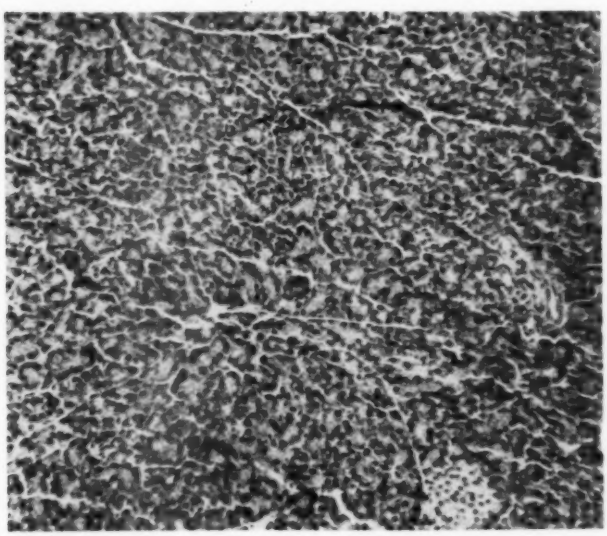


Fig. 9.—Pancreas of control rat. Hematoxylin and eosin; × 125.

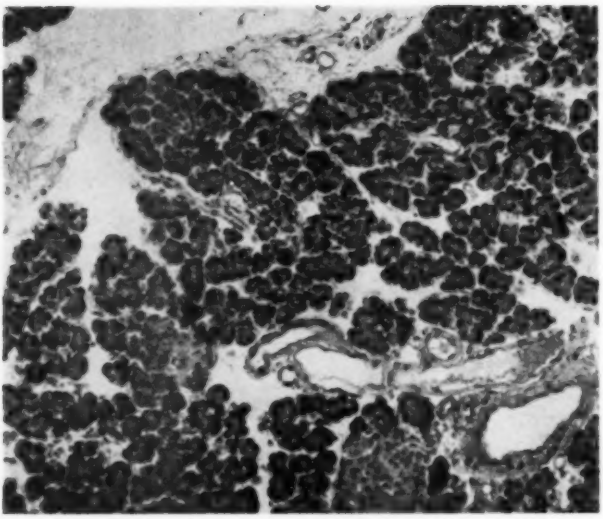


Fig. 10.—Pancreas of rat force-fed the threonine-devoid diet. Note the atrophy of the acini with edema of the stroma. Compare with Figure 9. Hematoxylin and eosin; × 125.

Pancreas: The pancreas was normal in the control animals (Fig. 9). In the threonine-devoid rats, the pancreas was enlarged and glistening and contained moderate to large amounts of clear edema fluid. On microscopic examination, the lobules of the pancreas were widely separated by pink-staining edema fluid. The acinar cells showed moderate loss of cytoplasm and of zymogen granules. The nuclei were prominent and were close together. The islets were normal (Fig. 10). Similar changes, but with less edema, were found in the rats force-fed histidine-devoid diets. In the animals given the methionine-devoid diet, the pancreas was grossly and microscopically normal.

Parotid Gland: In the animals of both the threonine- and the histidine-devoid groups, the parotid glands were smaller than normal (Figs. 11 and 12). On microscopic examination, the nuclei were much closer together than in the normal parotid gland (Figs. 13 and 14). This was due to the striking loss of cellular cytoplasm. The parotid glands of the control animals and of those given methionine-devoid diet were normal.

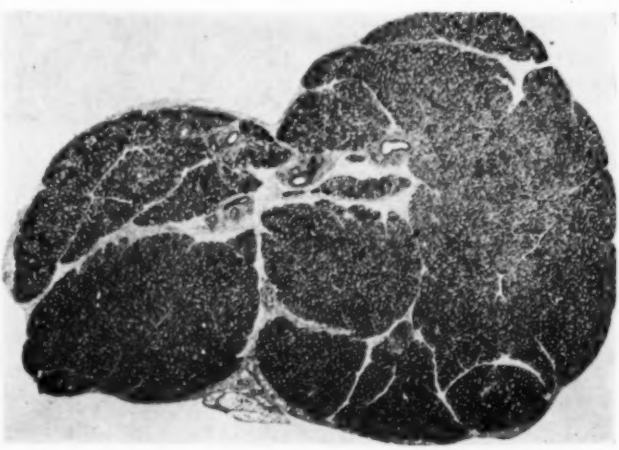


Fig. 11.—Parotid gland of control rat. Hematoxylin and eosin; reduced about 10% from mag. × 20.

Fig. 12.—Parotid gland of rat force-fed the histidine-devoid diet. Note the atrophy of the gland, with more compact acini. Compare with Figure 11. Hematoxylin and eosin; reduced about 10% from mag. × 20.

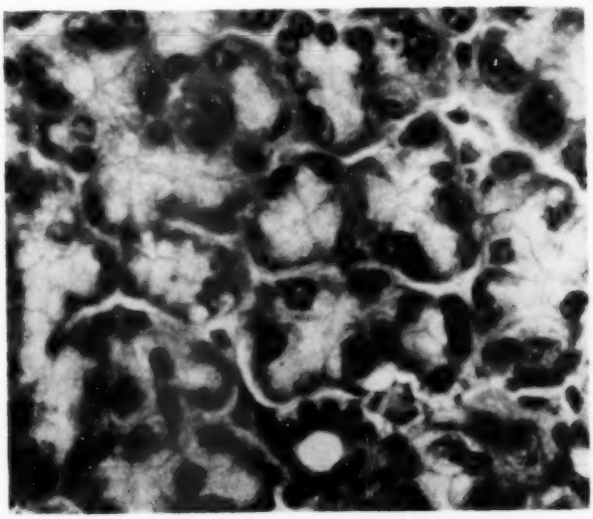


Fig. 13.—Parotid gland of control rat. Hematoxylin and eosin; × 670.

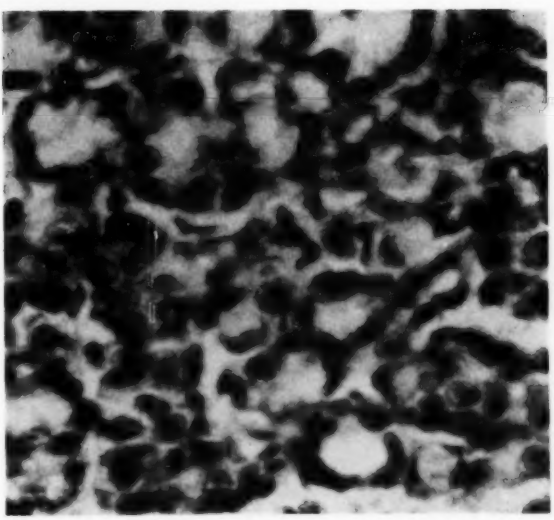


Fig. 14.—Parotid gland of rat force-fed the histidine-devoid diet. Note the atrophy of the acinar cells. Compare with Figure 13. Hematoxylin and eosin; × 670.

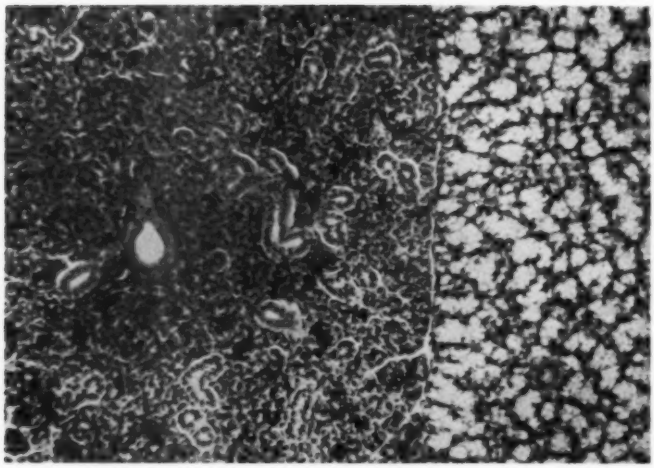


Fig. 15.—Submaxillary gland of control rat. The mucus portion of gland is on right. Hematoxylin and eosin; reduced about 10% from mag. × 125.

Submaxillary Gland: The submaxillary gland was normal in the control animals and in those of the methionine-devoid group (Fig. 15). In contrast, some animals given the threonine- or histidine-devoid diet had glands in which the mucus and serous cells showed atrophy of their cytoplasm (Fig. 16). In a few rats, the gland contained edema fluid and showed separation of the lobules. In general, the animals in the threonine-devoid group showed more severe submaxillary gland alterations than did those in the histidine-devoid group.

Adrenal Gland: The adrenal cortex was thinner in rats given the threonine- or histidine-devoid diet than in the control animals (Figs. 17 and 18). The zona glomerulosa was prominent and much more basophilic than in the normal gland. The prominence of this zone was due to both the loss of cellular cytoplasm and an increase in nuclear basophilia. In the methionine-devoid and control groups, the animals had normal adrenal glands.

Stomach: In animals given the control or methionine-devoid diets, the forestomach and glandular part of the stomach were

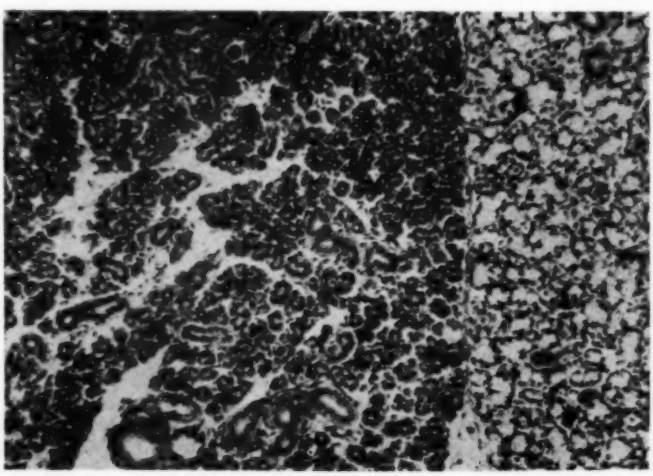


Fig. 16.—Submaxillary gland of rat force-fed the threonine-devoid diet. Note the atrophy of the serous and mucous glands and the edema of the stroma. Compare with Figure 15. Hematoxylin and eosin; reduced about 10% from mag. × 125.

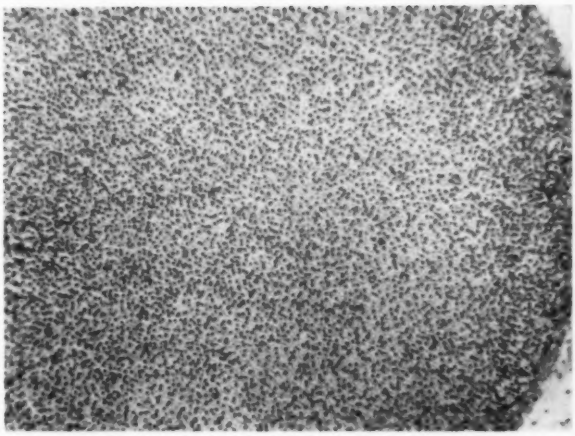


Fig. 17.—Adrenal cortex of control rat. Hematoxylin and eosin; × 95.

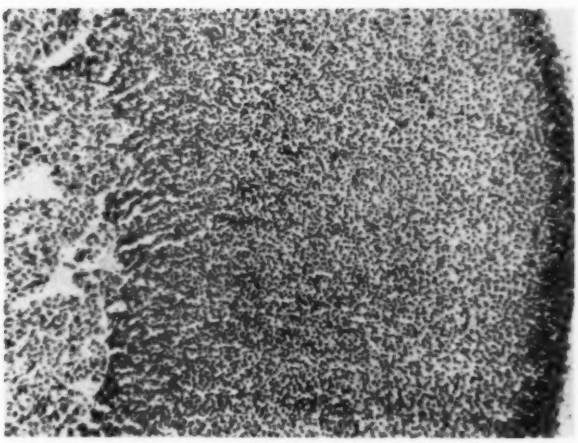


Fig. 18.—Adrenal cortex of rat force-fed the threonine-devoid diet. Note the prominence of the zona glomerulosa and the thinning of cortex. Compare with Figure 17. Hematoxylin and eosin; × 95.

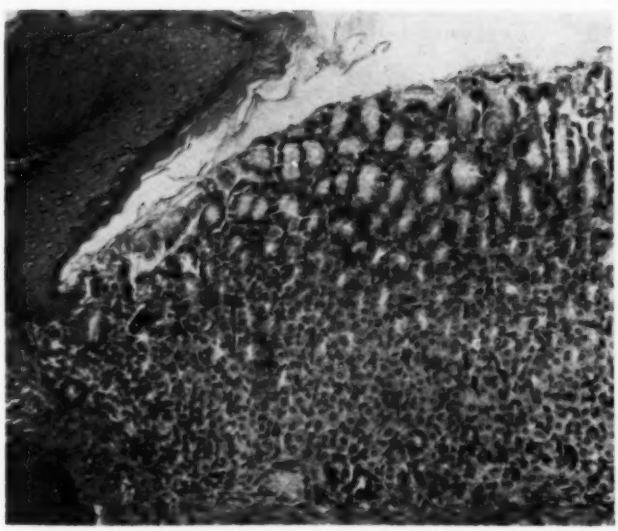


Fig. 19.—Stomach of control rat. Hematoxylin and eosin; × 125.

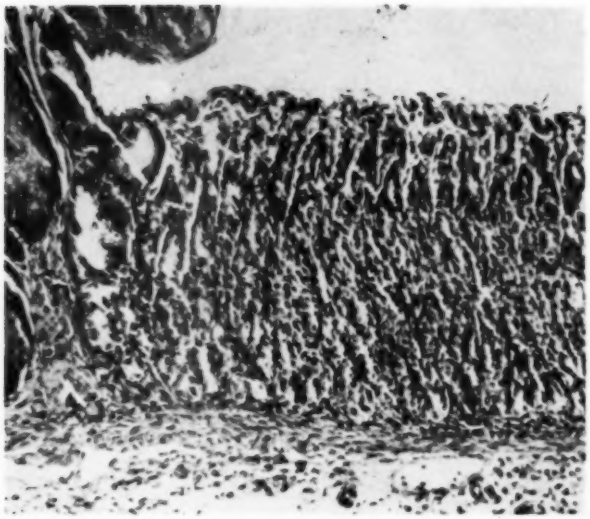


Fig. 20.—Stomach of rat force-fed the histidine-devoid diet. Note the atrophy of the glands. Compare with Figure 19. Hematoxylin and eosin; × 125.

normal (Fig. 19). Abundant mucinous material was demonstrable in the glands of the superficial mucosa with the periodic acid-Schiff stain (Fig. 21). In contrast, the superficial gastric glands in rats force-fed the threonine- or histidine-devoid diet showed atrophy with an almost complete absence of mucin (Figs. 20 and 22).

The following organs showed no gross or microscopic changes: heart, kidney, lung, and small intestine.

Three-Day Experiments.—In several force-feeding experiments, rats were killed after being given the control or threonine-devoid diet for three days. Sections of liver stained with Best's carmine revealed

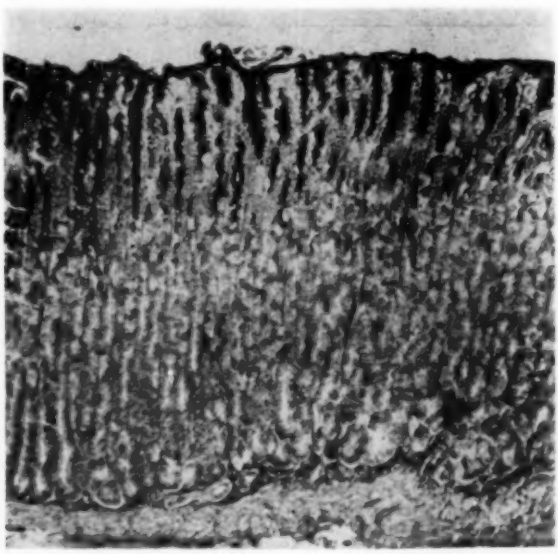


Fig. 21.—Stomach of control rat. Note the abundant mucin in the superficial glandular cells. Periodic acid-Schiff stain; × 125.

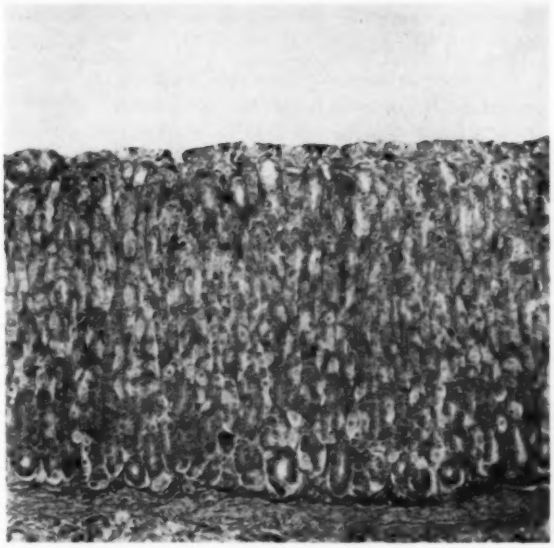


Fig. 22.—Stomach of rat force-fed the histidine-devoid diet. Note the small amount of mucin and the thinning of the glandular portion of stomach. Periodic acid-Schiff stain; × 125.

a moderate increase in hepatic glycogen in animals given the devoid diet. The pancreas showed early degenerative changes in the experimental animals. Other organs were essentially normal.

Comment

The results of this study clearly show that pathologic changes in the liver, pancreas, salivary glands, adrenal gland, and stomach can be rapidly induced in young rats when the animals are force-fed diets devoid of threonine or histidine. The changes with methionine-devoid diets were less striking. These observations are in obvious contrast to those obtained when the same diets were fed ad libitum. In the latter case, few if any histopathologic changes were found in short-term experiments.

There are many differences between force-feeding and ad libitum regimens. However, since the tissues in each control group were normal, the major difference must result from differences in the quantity rather than the quality of food taken. From the results of this study, it would appear that the amount of the diet consumed may be just as important as the composition of the diet in determining the tissue response of the animal.

It is a common experience in nutritional experiments that animals given diets deficient in one of many different essential food constituents consume progressively less food and therefore show increasing evidence of undernutrition. It is apparent from our study that the drastic reduction in food intake radically alters the morphologic reaction patterns of the tissues to deficient diets. This factor, however, has not been adequately emphasized in studies on amino acid deficiencies. Yet, a critical survey of the literature reveals the influence of this factor as strikingly as was seen in our study. For example, using force-feeding techniques, Adamstone and Spector [7] and Samuels and co-workers [8] described many obvious pathologic tissue changes, including periportal fatty liver, within a short period of time after the onset of feeding diets devoid of tryptophan, phenylalanine, or isoleucine. In contrast, many other investigators found far fewer specific tissue changes with diets deficient in single essential amino acids

when these were fed ad libitum for much longer periods of time.[9,10,20-27,30]

This same relationship between the quantity of food consumed and the occurrence of pathologic changes has also been emphasized by several investigators of human nutritional disease. For example, from their studies on kwashiorkor, in which periportal fatty liver and glandular-cell atrophy are outstanding tissue changes, Waterlow,[31] Davies,[32] and Brock and Autret[33] have stressed that a prominent feature of the dietary history in such conditions is the relatively high intake of carbohydrate coupled with inadequate protein. Also, Housden[34] has stated that "malnutrition and overfeeding go hand in hand." In contrast to these is the study of the effects of general undernutrition in Europe during the last war, in which no specific lesions of the liver were found despite the severe restriction of protein intake.[35]

The force-feeding in place of ad libitum feeding of deficient diets has another important consequence, namely, the induction of a greater degree of imbalance of dietary amino acids. Amino acid imbalances have been implicated by Elvehjem[6] in the pathogenesis of some forms of fatty liver.

The occurrence of a fatty liver with a periportal distribution in animals force-fed threonine-, methionine-, or histidine-devoid diets is of special interest. Until a few years ago, it was generally thought that the major role of dietary protein in the pathogenesis of fatty liver was the provision of methyl groups for choline synthesis.[36] However, increasing evidence has been presented in recent years to show that protein and amino acids have additional roles in the production and prevention of fatty liver. An important factor calling for such a reinterpretation has been the recognition that the lobular distribution of the excess liver lipid differs with different types of fatty livers. For example, with choline deficiency in the rat and mouse, the excess lipid first appears about the central vein and then progressively involves the remainder of the lobule. In contrast, a periportal lipid distribution is found in kwashiorkor in humans and in protein and amino acid deficiencies in rats.

Apparently, periportal fatty liver in rats given amino acid-devoid diets is not a function of the special role of any single essential amino acid, since it has been observed with deficiencies of tryptophan,[7,8,11] isoleucine,[8,11] and phenylalanine,[8,11] as well as threonine, methionine, and histidine. The results obtained with ethionine, which also induces a periportal fatty liver,[37] suggested that this disorder might be the result of interference with hepatic protein synthesis.[19] If true, this could explain why different essential amino acid deficiencies are capable of inducing the same type of fatty liver. However, investigation of protein synthesis in our study failed to show such a biochemical abnormality.[38] Therefore, the mechanism of the induction of this lesion with different amino acids still remains unexplained.

The pathologic findings in rats force-fed diets devoid in some single essential amino acids appear to be remarkably similar to those seen in kwashiorkor.[13] Kwashiorkor is probably due to an inadequate intake of high-quality protein and can apparently be cured by the administration of sufficient amounts of good quality protein[13] or of properly balanced amino acid mixtures.[39] Pathologically, pancreatic and salivary gland atrophy and periportal fatty liver are prominent findings. It is probably more than just coincidence that the tissue reactions in the rat force-fed diets devoid in one of several essential amino acids should resemble so closely those in human beings with kwashiorkor. It is very likely that the amino acid-devoid diets when fed in "adequate" amounts by stomach tube produce acute experimental models of the more complex human disorder.

Summary

Rats force-fed purified diets devoid in threonine or histidine were found to de-

velop a periportal fatty liver, excess hepatic glycogen, pancreatic edema and atrophy, and atrophy of salivary glands, gastric mucosa, and adrenal cortex within seven days after beginning the diets. Rats force-fed a methionine-devoid diet showed periportal fatty liver but few of the other pathologic changes. Animals fed the same diets ad libitum, in contrast to those force-fed, consumed less food and showed no specific pathologic changes. The differences in the results between the force-feeding and the ad libitum feeding regimens are explained in terms of the different quantity of the deficient diets consumed. The lesions observed in the rats force-fed threonine- or histidine-devoid diets resembled those reported to be characteristic of kwashiorkor.

The Dow Chemical Company, through Dr. R. W. Colby, supplied many of the amino acids used in this study. Some of the lysine and methionine was supplied by E. I. du Pont de Nemours and Company, through Dr. N. W. Flodin.

Tulane University School of Medicine, Department of Pathology, 1430 Tulane Ave. (12) (Dr. Farber).

REFERENCES

1. Wang, C. F.; Hegsted, D. M.; Lopi, A.; Zamcheck, N., and Black, M. B.: Progressive Changes in Liver Composition, Function, Body Fluids, and Liver Cytology During Protein Depletion in the Rat and the Effect of Choline upon These Changes, J. Lab. & Clin. Med. 34:953-964, 1949.

2. Jaffe, E. R.; Humphreys, E. M.; Benditt, E. P., and Wissler, R. W.: Effects of Various Degrees of Protein Depletion on Histologic and Chemical Structure of Rat Liver, Arch. Path. 47:411-423, 1949.

3. Best, C. H.; Hartroft, W. S., and Lucas, C. C.: Effects of Dietary Protein, Lipotropic Factors, and Re-Alimentation on Total Hepatic Lipids and Their Distribution, Brit. M. J. 1:1439-1444, 1955.

4. Lucas, C. C., and Ridout, J. H.: The Lipotropic Activity of Protein, Canad. J. Biochem. & Phyiol. 33:25-30, 1955.

5. Shils, M. E.; Friedland, I., and Stewart, W. B.: The Rapid Development of Portal Fatty Liver in Rats Consuming Various Plant Materials, Proc. Soc. Exper. Biol. & Med. 87:473-476, 1954.

6. Elvehjem, C. A.: Amino Acid Balance in Nutrition, J. Am. Dietet. A. 32:305-308, 1956.

7. Adamstone, F. B., and Spector, H.: Tryptophan Deficiency in the Rat: Histologic Changes Induced by Forced Feeding of Acid-Hydrolyzed Casein Diet, Arch. Path. 49:173-184, 1950.

8. Samuels, L. T.; Goldthorpe, H. C., and Dougherty, T. F.: Metabolic Effects of Specific Amino Acid Deficiencies, Fed. Proc. 10:393, 1951.

9. Dick, F., Jr.; Hall, W. K.; Sydenstricker, V. P.; McCollum, W., and Bowles, L. L.: Accumulation of Fat in the Liver with Deficiencies of Threonine and of Lysine, A. M. A. Arch. Path. 53:154-159, 1952.

10. Cole, A. S., and Scott, P. P.: Tissue Changes in the Adult Tryptophan-Deficient Rat, Brit. J. Nutrition 8:125-138, 1954.

11. Van Pilsum, J. F.; Speyer, J. F., and Samuels, L. T.: Essential Amino Acid Deficiency and Enzyme Activity, Arch. Biochem. 68:42-53, 1957.

12. Forbes, R. M., and Vaughan, L.: Nitrogen Balance of Young Albino Rats Force-Fed Methionine- or Histidine-Deficient Diets, J. Nutrition 52:25-37, 1954.

13. Trowell, H. C.; Davies, J. N. P., and Dean, R. F. A.: Kwashiorkor, London, Edward Arnold & Co., 1954.

14. Davies, J. N. P.: The Essential Pathology of Kwashiorkor, Lancet 1:317-320, 1948.

15. Hartroft, W. S.: Accumulation of Fat in Liver Cells and Lipodiastaemata Preceding Experimental Dietary Cirrhosis, Anat. Rec. 106:61-87, 1950.

16. Waterlow, J.: Enzyme Activity in Human Liver, in Liver Injury: Transactions of the 11th Conference, April 30 and May 1, 1952, New York, edited by F. W. Hoffbauer, New York, Josiah Macy, Jr. Foundation, 1952, pp. 72-110.

17. Burch, H. B.; Arroyave, G.; Schwartz, R.; Padilla, A. M.; Béhar, M.; Viteri, F., and Scrimshaw, N. S.: Biochemical Changes in the Liver Associated with Kwashiorkor, J. Clin. Invest. 36:1579-1587, 1957.

18. Farber, E.: Protein Deficiency and Fatty Liver, Bull. Tulane Univ. M. Fac. 14:71-80, 1955.

19. Farber, E.: Relationship of Disturbed Hepatic Protein Metabolism to Fatty Liver Induced by Ethionine, Fed. Proc. 14:402, 1955.

20. Maun, M. E.; Cahill, W. M., and Davis, R. M.: Morphologic Studies of Rats Deprived of Essential Amino Acids: I. Phenylalanine, Arch. Path. 39:294-300, 1945.

21. Maun, M. E.; Cahill, W. M., and Davis, R. M.: Morphologic Studies of Rats Deprived of Essential Amino Acids: II. Leucine, Arch. Path. 40:173-178, 1945.

22. Maun, M. E.; Cahill, W. M., and Davis, R. M.: Morphologic Studies of Rats Deprived of Essential Amino Acids: III. Histidine, Arch. Path. 41:25-31, 1946.

23. Schwartz, C.; Scott, E. B., and Ferguson, R. L.: Histopathology of Amino Acid Deficiencies: I. Phenylalanine, Anat. Rec. 110:313-327, 1951.

24. Scott, E. B., and Schwartz, C.: Histopathology of Amino Acid Deficiencies: II. Threonine, Proc. Soc. Exper. Biol. & Med. 84:271-276, 1953.

25. Scott, E. B.: Histopathology of Amino Acid Deficiencies: III. Histidine, A. M. A. Arch. Path. 58:129-141, 1954.

26. Scott, E. B.: Histopathology of Amino Acid Deficiencies: IV. Tryptophan, Am. J. Path. 31: 1111-1129, 1955.

27. Scott, E. B.: Histopathology of Amino Acid Deficiencies: V. Isoleucine, Proc. Soc. Exper. Biol. & Med. 92:134-140, 1956.

28. Hegsted, D. M.; Mills, R. C.; Elvehjem, C. A., and Hart, E. B.: Choline in the Nutrition of Chicks, J. Biol. Chem. 138:459-466, 1941.

29. Shay, H., and Gruenstein, M.: A Simple and Safe Method for the Gastric Instillation of Fluids in the Rat, J. Lab. & Clin. Med. 31:1384-1386, 1946.

30. Glynn, L. E.; Himsworth, H. P., and Neuberger, A.: Pathologic States Due to Deficiency of the Sulfur-Containing Amino Acids, Brit. J. Exper. Path. 26:326-337, 1945.

31. Waterlow, J. C.: Fatty Liver Disease in Infants in the British West Indies, Medical Research Council, Special Report Series No. 263, London, Her Majesty's Stationery Office, 1948.

32. Davies, J. N. P.: Nutrition and Nutritional Diseases, Ann. Rev. Med. 3:99-132, 1952.

33. Brock, J. F., and Autret, M.: Kwashiorkor in Africa, World Health Organization, Monograph Series No. 8, New York, Columbia University Press, 1952.

34. Housden, L.: Infant Feeding in North-East Africa, Brit. M. J. 2:456-457, 1950.

35. Studies of Undernutrition, Wuppertal, 1946-9, Medical Research Council, Special Report Series No. 275, London, Her Majesty's Stationery Office, 1951.

36. Best, C. H.: Protection of Liver and Kidneys by Dietary Factors: Choline and Its Precursors as Lipotropic Agents, Fed. Proc. 9: 506-511, 1950.

37. Koch-Weser, D.; Farber, C., and Popper, H.: Fatty Liver With and Without Necrosis: Histological and Biochemical Investigations, A.M.A. Arch. Path. 51:498-509, 1951.

38. Sidransky, H., and Farber, E.: Chemical Pathology of Acute Amino Acid Deficiencies: II. Biochemical Changes in Rats Fed Threonine- or Methionine-Devoid Diets, A. M. A. Arch. Path., this issue, pp. 135-158.

39. Hansen, J. D. L.; Howe, E. E., and Brock, J. F.: Amino-Acids and Kwashiorkor, Lancet 2: 911-913, 1956.

Chemical Pathology of Acute Amino Acid Deficiencies

II. Biochemical Changes in Rats Fed Threonine- or Methionine-Devoid Diets

HERSCHEL SIDRANSKY, M.D., and EMMANUEL FARBER, M.D., Ph.D., New Orleans
With the Technical Assistance of Mary Amelia Anderson

The purposes of this investigation as outlined in the preceding communication [1] were to study the morphologic and biochemical changes occurring in the liver, pancreas, and other organs of rats force-fed purified diets devoid in certain single essential amino acids. This paper presents some aspects of protein, lipid, and carbohydrate metabolism in the liver, pancreas, and skeletal muscle in animals force-fed diets devoid in threonine or methionine as compared to animals fed the same diets ad libitum. The gross and microscopic pathologic changes in the same animals have previously been described.[1] The biochemical, as well as the pathologic, changes were very different in the animals force-fed the devoid diets from the changes in those fed the same diets ad libitum. The most surprising biochemical observation in these animals was the failure to detect any general interference with hepatic protein synthesis in the rats force-fed the devoid diets, despite the appearance of pathologic changes in the liver.

Experimental Data

In the experiments with immature rats, the animals and diets were those described in the previous paper.[1] Briefly, the animals were fed by stomach tube or fed ad libitum diets composed of pure amino acids, sucrose, corn oil, salt mixture, cod liver oil, and a complete vitamin mixture. Some animals were fed this diet with a full complement of amino acids, while others were fed the same diet but devoid of threonine or methionine. Animals were killed after either three days or seven days of these diets.

In two additional experiments, adult male and female Sprague-Dawley rats weighing between 180 and 195 gm. were used rather than immature animals. These rats were force-fed the same purified diet as was given the immature rats in daily amounts of 0.7 gm. of diet per 10 gm. of initial body weight and were killed after three days. The results in these experiments are expressed in terms of amount per organ per 100 gm. body weight.

Liver.—Pieces of liver from two major lobes were rapidly removed, weighed, and placed in either 30% KOH or homogenized in 5% trichloroacetic acid (TCA) for glycogen determination. Another weighed piece was homogenized in ice-cold distilled water, and suitable aliquots were added to an equal volume of cold 10% TCA for protein, protein-radioactivity, and nucleic acid determinations. One large piece of liver was weighed and then frozen at −15 C for subsequent lipid and lipid-radioactivity measurements.

Pancreas.—The pancreas was rapidly removed *in toto*, chilled, and weighed. Care was taken to exclude surrounding adipose tissue and lymph nodes. Part of the organ was homogenized in ice-cold 0.02 M phosphate buffer, pH 7.6, and suitable aliquots were added to an equal volume of cold 10% TCA for protein and protein-radioactivity determinations. The remainder was stored at −15 C.

Gastrocnemius Muscle.—The right gastrocnemius muscle was carefully removed, chilled, weighed, and homogenized in cold distilled water. An aliquot was added to an equal volume of cold 10% TCA for protein determinations.

Protein Determination.—Protein was measured by micro-Kjeldahl determination [2] on the TCA precipitates from suitable aliquots of the liver, pancreas, and gastrocnemius muscle. The aliquots were washed in succession with 5% TCA, 95% ethanol, ethanol-ethyl ether mixture (3:1), and

Submitted for publication March 20, 1958.

Departments of Pathology and Biochemistry, Tulane University School of Medicine. Life Insurance Medical Research Fellow 1956-1957 (Dr. Sidransky).

Supported in part by research grants from the National Institute of Arthritis and Metabolic Diseases, United States Public Health Service (A-610), and the Life Insurance Medical Research Fund.

ethyl ether. This precipitate was digested, and ammonia was measured by Nesslerization after precipitating some of the Hg catalyst with Na_2S and removing the H_2S by boiling.

Lipid Analysis.—For total liver lipid, the frozen aliquot of liver was thawed, ground to a dry powder with anhydrous sodium sulfate, and extracted with chloroform for 24 hours. After evaporation of the chloroform, the residue was extracted with petroleum ether and the lipid remaining on evaporation of this solvent was weighed. Aliquots of this lipid residue dissolved in chloroform were used for determinations of cholesterol and phospholipid. Cholesterol was determined by the method of Carr and Drekter,[3] and phospholipid, by digesting and measuring P by the method of Fiske and Subbarow.[4]

Amino Acid Incorporation.—In these experiments, the procedure was the same as that described previously, with the addition of the intraperitoneal injection of the radioactive amino acid three hours before killing each animal. In one experiment, an aqueous solution of 0.05 mg. of L-leucine, biologically labeled with C^{14}, was administered. This dose contained 3.37×10^6 measured counts per minute. In another experiment, the administered solution contained 0.26 mg. of L-valine, biologically labeled with C^{14} and having 3.68×10^6 counts per minute. In the third experiment, 0.059 mg. of L-isoleucine, biologically labeled with C^{14} and containing 3.70×10^6 counts per minute, was used. The radioactive amino acids were obtained from the Nuclear Instrument and Chemical Corporation on allocation from the United States Atomic Energy Commission. Aliquots of liver and pancreas homogenates and of plasma were precipitated with 10% TCA and were subsequently washed four times with 5% TCA and once each with 95% ethanol, ethanol-ethyl ether mixture (3:1), and ethyl ether. Five milligrams of the corresponding nonradioactive amino acid were added to the second and third TCA washes. The dried protein powder was plated on Whatman No. 50 filter paper and counted in a windowless flow counter to a precision of 3%. The quantity of protein in the counted sample was determined by the micro-Kjeldahl method and Nesslerization. The counts were corrected to 0 mg. by a self-absorption curve. The total radioactivity in the first two TCA washes (acid-soluble radioactivity) was determined by plating aliquots on stainless-steel planchets after concentrating the solution to a small volume (5 ml.). For liver lipid radioactivity measurement, a weighed aliquot of extracted lipid (see above) was diluted with chloroform. A measured quantity was then plated on stainless-steel planchets and counted.

Enzyme Studies.—In several experiments aliquots of fresh liver were used to determine glucose-6-phosphatase activity by the method of Cori and Cori.[6]

The response of liver tryptophan peroxidase activity to the administration of tryptophan was determined in a few experiments. Three milliliters of an aqueous solution containing 225 mg. of DL-tryptophan was injected intraperitoneally into animals of the control and the threonine-, and methionine-devoid groups on the seventh morning of the force-feeding experiments. Control rats of the experimental and control groups received 3 ml. of saline in a similar manner. The animals were killed five hours after the injections. All rats had received their last feeding 18 hours before the injection. Aliquots of liver were homogenized in cold 0.14 M KCL solution, and the enzyme assay was performed by the method of Knox and Auerbach.[6] The results are expressed as micromoles of kynurenine per liver per hour.

Aliquots of the pancreatic homogenates were analyzed for amylase activity by the method of Smith and Roe.[7] The results are expressed as Smith and Roe units per pancreas. In one seven-day force-feeding experiment, amylase resynthesis after depletion with pilocarpine was measured as previously described.[8] In this experiment water was removed from the cages in the morning 18 hours after last feeding, and the rats were given intramuscular injections of 3 mg. of pilocarpine HCl. Eight hours later, the animals were killed and pancreatic amylase was determined.

Other Determinations.—Liver glycogen was determined on approximately 1 gm. aliquots of liver by the methods of Seifter et al.[9] and Carroll et al.[10] Blood glucose was determined on tungstic acid filtrates by the anthrone method of Seifter et al.[9] Liver, pancreas, gastrocnmius muscle, and plasma nonprotein amino acid nitrogen were determined on supernatant solutions after tungstic acid precipitation of protein. The method used was that of Frame, Russel, and Wilhelmi,[11] as modified by Russel.[12] Ribonucleic acid (RNA) and deoxyribonucleic acid (DNA) determinations were performed by the method of Schmidt and Thannhauser,[13] using measurement both of P^{32} and of ultraviolet absorption, as described by Logan et al.[14]

Results

Immature Rats Force-Fed Threonine-Devoid Diet for Seven Days

In a previous paper,[1] the changes in body and organ weights in these animals were reported. The animals in the control group gained weight, while those in the threonine-devoid group lost a small amount of weight. However, the liver and pancreas weights

TABLE 1.—*Protein and Nonprotein Amino Acid Nitrogen Content of Liver, Plasma, and Pancreas of Immature Rats Force-Fed Threonine-Devoid or Methionine-Devoid Diets* *

Determination	Group †	Liver, Mg/Liver	Plasma, Mg/Ml.	Pancreas, Mg/Pancreas
Protein	C	(16) 633±16 ‡	(2) 74.2±9.9 ‡	(14) 52.3±5.1 ‡
	TD	(16) 677±23 §	(4) 70.8±7.1 §	(16) 58.8±2.3 §
	C	(9) 599±22	(3) 96.0±8.6	(6) 58.8±10.4
	MD	(9) 606±33 §	(2) 97.6±4.2 §	(6) 68.4±5.9 §
Nonprotein Amino acid N	C	(6) 3.86±0.48	(3) 0.106±0.004	(3) 0.364±0.054
	TD	(7) 5.79±0.56 ‖	(4) 0.081±0.009 §	(4) 0.352±0.031 §
	MD	(6) 3.45±0.26 §	(2) 0.130±0.012 §	(3) 0.381±0.056 §

* Number in parentheses indicates number of animals in group.
† C indicates control; TD, threonine-devoid; MD, methionine-devoid.
‡ Mean value ± standard error of the mean.
§ $P > 0.05$ (not significant).
‖ P between 0.01 and 0.05 (probably significant).

of the experimental rats were significantly greater than those of the controls.

Liver.—Protein Metabolism: The protein and nonprotein amino acid nitrogen content of the livers of the animals in the threonine-devoid and control groups are presented in Table 1. It is noteworthy that the liver protein content of animals in the threonine-devoid group was the same as, or slightly greater than, that in the animals of the control group. The nonprotein amino acid nitrogen content was considerably elevated over that in the controls.

Protein synthesis in the liver, as measured by radioactive amino acid incorporation into liver protein and plasma protein, was also not decreased in the rats force-fed the threonine-devoid diet (Table 2). In fact, in three experiments, each with use of a different radioactive amino acid, increased amino acid incorporation was apparent in animals fed threonine-devoid diets. The results are expressed as radioactivity per total liver protein rather than as specific activity (radioactivity per unit weight of protein) and are therefore corrected for any differences in the size of the non-radioactive-protein pool diluting the radioactive proteins. This increased radioactivity in the protein within a defined time interval could be due to (*a*) increased specific activity of the precursor free amino acid with no change in the actual rate of incorporation of the amino acid into protein; (*b*) increased rate of incorporation of the amino acid into protein with equal or decreased specific activity of the precursor free amino acid, or (*c*) a combination of (*a*) and (*b*). Ideally, in order to interpret the differences in the radioactivity in the protein, one should know the specific activity of the precursor amino acid at the time of incorporation. Since this changes with time, an integrated value over the time allowed for incorporation would be desirable. To obtain this value would have necessitated the killing of many animals at very short periods of time after the administration of the radioactive amino acid. In lieu of this, the total nonprotein acid-soluble radioactivity was determined at the time of killing. Total liver protein radioactivities are therefore corrected for this value in all rats. These corrected values are still higher in animals in the threonine-devoid group than in the controls. A second calculation is made to correct for differences in the liver nonprotein amino acid nitrogen content, considered to represent the amino acids available for incorporation. With this second correction, the total liver protein radioactivity is still significantly elevated in the experimental animals over that in the controls.

Since most plasma proteins are synthesized by the liver, radioactive amino acid incorporation into plasma protein was also measured in two experiments (Table 2). The results are expressed in terms of specific activity, rather than total protein radioactivity, since the total plasma volume was not known. The specific activity of the plasma proteins was elevated in the animals

TABLE 2.—*Incorporation of Radioactive Amino Acids into Protein of Liver, Pancreas, and Plasma of Immature Rats Force-Fed Threonine-Devoid or Methionine-Devoid Diets* *

Radioactive Amino Acid Administered	Group †	Liver Protein: A Total Radioactivity, CPM×10⁻³ ‡	Liver Protein: B A Corrected for Acid-Soluble Radioactivity, CPM §	Liver Protein: C B Corrected for Amino Acid N, CPM ‖	Pancreatic Protein: A Total Radioactivity, CPM×10⁻³ ‡	Pancreatic Protein: B A Corrected for Acid-Soluble Radioactivity, CPM §	Pancreatic Protein: C B Corrected for Amino Acid N, CPM ‖	Plasma Protein: A Specific Activity, CPM/Mg. Protein	Plasma Protein: B A Corrected for Liver Acid-Soluble Radioactivity, CPM Mg. Protein §	Plasma Protein: C B Corrected for Liver Amino Acid N, CPM/Mg. Protein ‖
L-Leucine	C	(4) 65.5±19.5 ¶	(3) 6.8±0.3 ¶	..	(4) 7.3±0.7 ¶	(4) 4.1±0.6 ¶	..	..	..	..
	TD	(3) 160.0±8.3	(3) 9.3±0.4	..	(3) 8.5±1.2	(3) 3.8±0.2	..	..	..	..
L-Valine	C	(3) 86±7	(2) 10.4±1.1	(2) 54±5 ¶	(3) 19.7±3.1	(3) 19.2±5.0	..	(3) 255±60 ¶	(2) 0.025±0.004 ¶	(2) 0.129±0.022 ¶
	TD	(3) 199±31	(3) 15.8±1.8	(3) 108±18	(3) 17.9±2.6	(3) 13.3±1.9	..	(3) 509±26	(3) 0.040±0.002	(3) 0.287±0.029
	MD	(3) 115±2	(3) 13.3±1.8	(3) 53±7	(3) 47.7±11.9	(3) 37.9±11.0	..	(3) 554±150	(3) 0.060±0.007	(3) 0.236±0.030
L-Isoleucine	C	(3) 83±7	(3) 5.7±0.4	(3) 16.7±3.0	(3) 42.6±10.0	(3) 16.4±1.4	(3) 6.11±1.35 ¶	(3) 145±27	(3) 0.010±0.000	(3) 0.029±0.003
	TD	(4) 288±12	(4) 9.3±0.9	(4) 44.9±6.5	(4) 27.6±1.7	(4) 10.2±0.4	(4) 3.57±0.27	(4) 216±18	(4) 0.009±0.001	(4) 0.042±0.005
	MD	(3) 173±35	(3) 9.1±2.2	(3) 26.1±5.3	(3) 58.7±17.1	(3) 24.8±4.6	(3) 9.03±0.78	(2) 148±5	(2) 0.007±0.001	(2) 0.021±0.001

* Number in parentheses indicates number of animals in group.
† C indicates control; TD, threonine-devoid; MD, methionine-devoid.
‡ Radioactivity in total organ protein.
§ Total radioactivity or specific activity corrected for acid soluble radioactivity:

$$\left(\frac{\text{Total radioactivity in organ protein or specific activity}}{\text{Radioactivity in acid-soluble fraction of total organ}}\right).$$

‖ Corrected for difference in amino acid nitrogen in organ:

$$\left(\left[\frac{\text{Total radioactivity in organ protein or specific activity}}{\text{Radioactivity in acid-soluble fraction of total organ}}\right]\times\left[\text{nonprotein amino acid N per total organ}\right]\right).$$

¶ Mean value ± standard error of the mean.

TABLE 3.—*Liver and Pancreas Enzyme Studies of Immature Rats Force-Fed Threonine-Devoid or Methionine-Devoid Diets* *

Group	Liver, Tryptophan Peroxidase		Pancreas, Amylase	
	Unstimulated, Units/Liver †	Stimulated, Units/Liver ‡	Basal Level, Units/Pancreas	Resynthesis Level, Units/Pancreas §
Control	(2) 9.8	(2) 68.4	(13) 5,090	(3) 6,928
Threonine-devoid	(3) 7.2	(3) 44.0	(16) 2,290	(2) 4,704
Control	(2) 10.3	(3) 34.9	(9) 7,133	(3) 6,928
Methionine-devoid	(2) 9.3	(3) 34.3	(9) 8,574	(3) 6,071

* Number in parentheses indicates number of animals in group.
† Rats received saline intraperitoneally 5 hours before killing.
‡ Rats received tryptophan solution intraperitoneally 5 hours before killing.
§ Rats received pilocarpine intramuscularly 8 hours before killing.

in the threonine-devoid group over that in the controls. After correcting these specific activity values for liver acid-soluble radioactivity and liver nonprotein amino acid nitrogen as described above, the corrected values still show an elevation in the experimental animals over that in the controls.

As a check on the results of the radioactive experiments, the ability of the livers of some experimental and control rats to synthesize the adaptive enzyme system, tryptophan peroxidase, was measured in one experiment. Rats of each group responded to the intraperitoneal DL-tryptophan injection by an elevation in liver tryptophan peroxidase activity (Table 3). In the control group, the animals receiving DL-tryptophan showed a 598% increase in enzyme activity over those given injections of saline. In the threonine-devoid group under identical conditions, the rats given injections of DL-tryptophan showed a 513% increase in enzyme activity. The difference between the rise in enzyme activity in the two groups is very small (17%). This indicates that the experimental animals had about the same ability to synthesize this adaptive enzyme as did the control rats.

Lipid Metabolism: Table 4 summarizes the liver lipid analyses of the animals in the threonine-devoid and the control groups. The total liver lipid content was significantly greater in the experimental than in the control animals. Similar significant changes were also found in the liver cholesterol, phospholipid, and neutral fat. The neutral fat values were calculated by subtracting the cholesterol and phospholipid values from the total liver lipid and therefore were derived only in those animals in which cholesterol and phospholipid were determined.

In one seven-day experiment, analyses of the liver lipid iodine numbers and saponification numbers were performed. The control rats had a mean iodine number of 93.1, and the threonine-devoid, a mean value of 89.8. The saponification number of the lipid from the control animals was 189.7, and from the animals given the threonine-devoid diet it was 200.6.

Samuels and co-workers [15] suggested that the increased liver lipid in animals force-fed tryptophan-, isoleucine-, or phenylalanine-devoid diets might be due to an enhanced conversion of amino acids to lipid. In order to learn whether this reaction

TABLE 4.—*Lipid Analysis of Liver of Immature Rats Force-Fed Threonine-Devoid or Methionine-Devoid Diets* *

Group	Total Lipid, Mg/Liver	Cholesterol, Mg/Liver	Phospholipid, Mg/Liver	Neutral Fat (by Difference), Mg/Liver †
Control	(30) 163±7 ‡	(7) 10.0±0.5 ‡	(7) 81.8±5.4 ‡	(7) 66.6±11.0 ‡
Threonine-devoid	(34) 275±12 §	(9) 13.6±1.1 §	(9) 114.5±4.3 §	(9) 137.9±18.2 §
Control	(22) 170±8	--	--	--
Methionine-devoid	(21) 248±27 §	--	--	--

* Number in parentheses indicates number of animals in group.
† Neutral fat = total liver lipid—(cholesterol plus phospholipid) (see text for explanation).
‡ Mean value ± standard error of the mean.
§ $P<0.01$ (highly significant).

TABLE 5.—*Incorporation of Radioactivity from Labeled Amino Acids in Total Liver Lipid in Immature Rats Force-Fed Threonine-Devoid or Methionine-Devoid Diets*

Radioactive Amino Acid Administered	Group *	No. of Rats	Total Liver Lipid		
			A Total Radioactivity, CPM×10⁻³ †	B A Corrected for Acid-Soluble Radioactivity, CPM ‡	C B Corrected for Amino Acid N, CPM §
L-Leucine	C	3	0.79±0.04 ‖	0.080±0.003 ‖	--
	TD	3	0.90±0.06	0.055±0.009	--
L-Valine	C	2	0.91±0.01	0.114±0.000	0.593±0.000 ‖
	TD	3	1.27±0.55	0.102±0.017	0.707±0.072
	MD	3	1.14±0.27	0.126±0.019	0.500±0.080
L-Isoleucine	C	3	1.74±0.14	0.120±0.010	0.345±0.041
	TD	3	1.91±0.27	0.083±0.014	0.392±0.064
	MD	3	3.11±0.73	0.153±0.011	0.457±0.073

* C indicates control; TD, threonine-devoid; MD, methionine-devoid.
† Radioactivity in total liver lipid.
‡ Total radioactivity corrected for acid-soluble radioactivity: Total radioactivity in total liver lipid/radioactivity in acid-soluble fraction of total liver.
§ Corrected for differences in amino acid N in liver:

$$\left(\left[\frac{\text{Total radioactivity in liver lipid (A)}}{\text{Radioactivity in acid-soluble fraction of total liver}}\right]\times\left[\text{nonprotein amino acid N per total liver}\right]\right).$$

‖ Mean value ± standard error of the mean.

could account for the increase in liver lipid in our experimental animals, conversion of radioactive amino acids to total liver lipid was measured. Table 5 summarizes the results in three experiments. The results are expressed in terms of total liver lipid radioactivity as well as lipid radioactivity corrected for liver acid-soluble radioactivity and for liver nonprotein amino acid nitrogen as described above. The results indicate that accelerated conversion of amino acids to lipids cannot be the major mechanism for the increased liver lipid content in animals force-fed threonine-devoid diets.

Carbohydrate Metabolism: In order to check the histologic finding of increased glycogen in the livers of animals given the threonine-devoid diet,[1] liver glycogen content was determined in several experiments. As seen from Table 6, there was a significant elevation in liver glycogen content in the animals of the threonine-devoid group over that in the control animals.

Since the elevated glycogen in the livers of the experimental rats resembled histologically that seen in certain forms of von Gierke's disease, it was decided to assay the liver for glucose-6-phosphatase activity. This enzyme has been reported to be very low in the hepatic form of glycogen-storage disease.[16] However, no significant difference in this enzyme activity was found between the animals in the devoid and the control groups (Table 6).

Blood glucose values were also investigated for possible alterations in the experimental animals. The control rats had a mean value of 106 mg. %, and the experimental, a mean value of 102 mg. %.

Nucleic Acids: In view of the increase in liver weight and possibly also in liver protein in the experimental animals in comparison with the controls, it became of importance to evaluate the number of cells in the livers of both groups. This can be determined by learning the number of nuclei present in each liver. Since liver deoxyribonucleic acid is exclusively present in the nuclei, and since all diploid nuclei of any one species have a fairly constant DNA

TABLE 6.—*Liver Glycogen and Glucose-6-Phosphatase in Immature Rats Force-Fed Threonine-Devoid or Methionine-Devoid Diets* *

Group	Liver	
	Glycogen, Mg/Liver	Glucose-6-Phosphatase, Mg. P Liberated/Liver/Hr. at 30 C
Control	(18) 45.2±5.2 †	(4) 42.4±4.6 †
Threonine devoid	(22) 121.8±9.3 ‡	(5) 46.3±1.7 §
Control	(9) 40.9±6.1	--
Methionine-devoid	(7) 29.9±7.3 §	--

* Number in parentheses indicates number of animals in group.
† Mean value ± standard error of the mean.
‡ $P<0.01$ (highly significant).
§ $P>0.05$ (not significant).

TABLE 7.—*Liver Ribonucleic and Deoxyribonucleic Acids in Immature Rats Force-Fed Threonine-Devoid or Methionine-Devoid Diets*

Group	Rats, No.	Liver	
		RNA, Mg. P/Liver	DNA, Mg. P/Liver
Control	10	3.80±0.09 *	0.88±0.04 *
Threonine-devoid	9	5.22±0.17 †	0.95±0.07 ‡
Control	6	3.75±0.14	0.93±0.06
Methionine-devoid	6	4.20±0.21 ‡	0.94±0.09 ‡

* Mean value ± standard error of the mean.
† $P<0.01$ (highly significant).
‡ $P>0.05$ (not significant).

content,[17] analysis of liver DNA content gives a measure of the number of hepatic nuclei and therefore a measure of the number of cells present in the liver, assuming no change in ploidy has occurred during the experiment. Liver DNA and RNA were determined in several experiments (Table 7).

The liver DNA content was essentially the same in animals of the threonine-devoid and the control groups. Since no obvious difference between the number of nuclei per liver cell was found on histologic examination of animals in each group,[1] this result must mean that the number of cells per liver was the same. In contrast, liver RNA content of the animals in the threonine-devoid group was significantly elevated over that in the control group.

Analysis of Major Components: Despite the fact that the control animals gained weight while the animals given the threonine-devoid diet did not, the mean liver weight of the animals in the control group was 3.10 gm. and in the devoid group, 4.21 gm. In an attempt to account for this 35.8% increase in liver weight in the experimental animals, an analysis of dry weight, protein, lipid, and glycogen of the livers of animals in two experiments was performed. The dry weight of the livers of the animals in the threonine-devoid group was 35.8% greater than in the controls. Of this increase in dry weight, protein accounted for 18%; liver lipid, for 44%, and liver glycogen, for 38%.

Pancreas.—The protein and nonprotein amino acid nitrogen content of the pancreas in experimental and control animals is shown in Table 1. It is apparent from this Table that both the protein levels and the "free" amino acid levels were essentially the same in the animals of the threonine-devoid and the control groups.

Pancreatic amylase determinations were performed in several experiments. The animals of the threonine-devoid group had significantly less amylase activity per pancreas than did the controls (Table 3). These results are, however, of dubious significance by themselves, since the pancreatic enzyme level is the resultant of at least two processes, i. e., synthesis and secretion.

In order to help decide whether or not interference with synthesis is important in the experimental animals, protein synthesis was studied in the pancreas. This was measured by (*a*) radioactive amino acid incorporation into pancreatic protein and (*b*) pancreatic amylase resynthesis after depletion with pilocarpine. The incorporation of radioactive valine or isoleucine but not of leucine into total pancreatic protein was 30% to 40% less in the animals in the threonine-devoid group than in those of the control group (Table 2). Consistent with this finding are the results from one experiment on amylase resynthesis after depletion by pilocarpine (Table 3). Eight hours after enzyme depletion by pilocarpine, the pancreas of the experimental animals contained 30% less enzyme activity than did that of the control rats. Although these results are not conclusive, they do suggest that the pancreas of animals force-fed threonine-devoid diets had a diminished ability to synthesize protein. The finding of a normal or increased pancreatic protein content in the animals given the devoid diet is not necessarily inconsistent with this conclusion, since some of the protein may be of exogenous origin accompanying the edema fluid.[1]

Skeletal Muscle.—The results obtained by analyzing the right gastrocnemius muscle of

TABLE 8.—*Wet and Dry Weights, Protein, and Nonprotein Amino Acid N of the Right Gastrocnemius Muscle of Immature Rats Force-Fed Threonine-Devoid or Methionine-Devoid Diets*

Group	Rats, No.	Right Gastrocnemius Muscle			
		Wet Weight, Mg.	Dry Weight, %	Protein, Mg/Muscle	Amino Acid N, Mg/Muscle
Control	5	541±16 *	28.5±1.5 *	89.5±3.3 *	0.469±0.006 *
Threonine-devoid	6	409±12 †	28.5±1.0	67.3±2.8 †	0.317±0.027 †
Methionine-devoid	3	409±27 †	26.2±0.8	69.7±4.0 †	0.327±0.022 †

* Mean value ± standard error of the mean.
† $P<0.01$ (highly significant).

rats in two experiments are summarized in Table 8. There is a significant decrease in muscle weight in the animals of the threonine-devoid group in comparison with the controls. The percentage dry weight is constant in both groups. The content of muscle protein and nonprotein amino acid nitrogen is significantly decreased in the threonine-devoid group in comparison with controls.

Blood.—In some experiments, several types of blood determinations were performed. In the animals tube-fed the threonine-devoid diet, the values were as follows: hematocrit 44.9%, hemoglobin 11.7%, plasma protein 7.4%, and plasma nonprotein amino acid N 0.081 mg. per milliliter. In the control animals, the values for similar determinations were as follows: hematocrit 48.4%, hemoglobin 12.3%, plasma protein 7.1%, and plasma nonprotein amino acid N 0.106 mg. per milliliter. Most of these values are similar in both groups, except that the plasma amino acid N concentration is lower in animals in the threonine-devoid group than in the controls.

In one experiment, plasma blood volumes were determined on several rats from each group. The mean value for the control rats and that for the threonine-devoid rats were equal.

Immature Rats Fed Threonine-Devoid Diet ad Libitum for Seven Days

The changes in body weight, diet consumption, and weights of the liver and pancreas in animals fed the threonine-devoid or control diets ad libitum were reported in the preceding paper.[1] In these ad libitum experiments, the experimental animals showed a diminution in all of these values except for the pancreas, which was larger than in the controls.

Table 9 summarizes the values of liver protein, lipid, and glycogen content; pancreatic protein content, and right gastrocnemius muscle wet weight and protein in the experimental and the control groups. The liver protein was significantly decreased in the animals of the threonine-devoid group as compared to those in the control group. The liver glycogen content was essentially the same in animals of the two groups. The liver lipid content was lower in the animals of the threonine-devoid than of the control groups. The gastrocnemius muscle was significantly less in wet weight and in protein

TABLE 9.—*Composition of Liver, Pancreas, and Right Gastrocnemius Muscle in Immature Rats Fed Threonine-Devoid or Methionine-Devoid Diets ad Libitum* *

Group	Liver			Pancreas	Right Gastrocnemius Muscle	
	Protein, Mg/Liver	Lipid, Mg/Liver	Glycogen, Mg/Liver	Protein, Mg/Pancreas	Wet Weight, Mg.	Protein, Mg/Muscle
Control	(11) 549±25 †	(6) 205±51 †	(6) 99±23 †	(3) 65.6±7.0 †	(5) 587±11 †	(4) 100.3±1.2 †
Threonine-devoid	(11) 362±25 §	(6) 102±9 ¶	(6) 128±23 ¶	(3) 36.5±2.7 §	(5) 367±5 §	(5) 66.5±2.0 §
Methionine-devoid	(7) 359±24 §	(3) 153±3 ¶	(3) 151±55 ¶	(3) 49.0±9.1 ¶	(5) 398±15 §	(5) 76.1±2.7 §

* Number in parentheses indicates number of animals in group.
† Mean value ± standard error of the mean.
§ $P<0.01$ (highly significant).
‖ P between 0.01 and 0.05 (probably significant).
¶ $P>0.05$ (not significant).

TABLE 10.—*Changes in Body Weight and Weight and Composition of Liver and Pancreas of Immature Rats Force-Fed Threonine-Devoid Diets for Three Days*

Group	Change in Body Weight, Gm.	Liver				Pancreas	
		Weight, Gm.	Protein, Mg/Liver	Lipid, Mg/Liver	Glycogen, Mg/Liver	Weight, Mg.	Protein, Mg/Pancreas
Control (10)*	+3.0	3.22±0.11 †	583±22 †	151±14 †	32.4±6.9 †	327±16 †	45.3±3.2 †
Threonine-devoid (10)	−1.0	3.80±0.15 ‡	604±17 ‖	207±19 §	68.4±18.3 ‖	368±20 ‖	54.8±3.4 ‖

* Number of animals in group.
† Mean value ± standard error of the mean.
‡ $P<0.01$ (highly significant).
§ P between 0.05 and 0.01 (probably significant).
‖ $P>0.05$ (not significant).

in the experimental animals than in the controls.

Immature Rats Force-Fed Threonine-Devoid Diet for Three Days

Since many significant and interesting changes were found in the threonine-devoid experiments of seven days' duration, it was of interest to learn how early many of these changes would occur. For this reason, several three-day experiments were performed. Table 10 summarizes these findings.

There was a significant elevation of liver weight and a suggestive increase in liver glycogen and lipid in animals given the threonine-devoid diet as compared to those given the control diet. The pancreatic weight and protein content were slightly but insignificantly elevated in the animals of the threonine-devoid group in comparison with the controls. The pancreatic amylase content was 4802 Smith and Roe units in the experimental animals and 5545 Smith and Roe units in the controls. These three-day results indicate that many of the changes found at seven days had already begun within three days.

Mature Rats Force-Fed Threonine-Devoid Diet for Three Days

In several experiments mature male and female rats were force-fed threonine-devoid diets for three days. The animals in the threonine-devoid group showed a significant elevation in liver weight, lipid, and glycogen over values for the control animals (Table 11). The results in males and females from each group were combined, since there were no differences between the sexes. Liver protein and pancreatic weight and protein showed insignificant differences between the animals of the two groups. Thus, the liver and pancreas of mature rats respond to the force-feeding of a threonine-devoid diet in the same manner as and somewhat more quickly than those of immature animals.

TABLE 11.—*Weight and Composition of Liver and Pancreas of Adult Rats Force-Fed Threonine-Devoid or Methionine-Devoid Diets* *

Group †	Sex	Liver				Pancreas	
		Weight, Gm/100 Gm. Body Weight	Protein, Mg/Liver/ 100 Gm. Body Weight	Lipid, Mg/Liver/ 100 Gm. Body Weight ‡	Glycogen, Mg/Liver/ 100 Gm. Body Weight	Weight, Mg/100 Gm. Body Weight	Protein, Mg/Pancreas/ 100 Gm. Body Weight
C	M&F	(10) 3.88±0.12 §	(10) 571±11 §	(10) 253±14 §	(10) 95±24 §	(6) 383±18 §	(6) 48.1±3.3 §
TD	M&F	(9) 5.19±0.23 ‖	(9) 587±15 ¶	(9) 419±19 ‖	(9) 292±50 ‖	(6) 414±13 ¶	(6) 43.1±1.4 ¶
C	M	(10) 4.28±0.17	(10) 687±43	(10) 275±28	(10) 109±19	(10) 318±14	(10) 48.2±3.1
MD	M	(9) 4.23±0.15 ¶	(5) 674±45 ¶	(9) 292±14 ¶	(7) 124±23 ¶	(8) 306±6 ¶	(8) 43.1±2.5 ¶
C	F	(13) 4.21±0.14	(9) 653±55	(10) 289±25	(10) 81±16	(10) 361±21	(9) 51.6±3.2
MD	F	(12) 4.76±0.20 #	(9) 657±76 ¶	(12) 587±98 ‖	(9) 122±11 #	(9) 353±20 ¶	(8) 50.0±2.6 ¶

* Number in parentheses indicates number of animals in group.
† C indicates control; TD, threonine-devoid; MD, methionine-devoid.
‡ Data from Sidransky and Farber.[18]
§ Mean value ± standard error of the mean.
‖ $<P0.01$ (highly significant).
P between 0.05 and 0.01 (probably significant).
¶ $>P$ 0.05 (not significant).

Immature and Mature Rats Force-Fed Methionine-Devoid Diets

Most of the determinations performed on animals in the threonine-devoid group were also done on animals force-fed methionine-devoid diets. The results in immature rats force-fed for seven days are presented in Tables 1 to 8.

Liver analyses revealed a significant increase of total liver lipid in the experimental animals as compared to the controls (Table 4). Other determinations relating to studies of liver protein metabolism, glycogen content, nucleic acid content, and radioactive amino acid incorporation into liver lipid showed no significant differences between control and experimental animals. This is in contrast to many of the differences found between animals in the control and the threonine-devoid groups.

The pancreas revealed some interesting differences. The protein content of the pancreas of the animals in the methionine-devoid group was slightly increased over that in the controls (Table 1). Also the basal amylase content of the pancreas of the experimental animals was elevated over that in the controls (Table 3). After resynthesis following depletion by pilocarpine, the amylase level was essentially the same in both groups (Table 3). Incorporation of labeled L-valine and L-isoleucine into pancreatic protein was more than 50% greater in the pancreas of the rats in the methionine-devoid groups than in the controls (Table 2). This suggestive increase in protein synthesis in the pancreas of animals given the methionine-devoid diet is reminiscent of the results in rats given ethionine in which an acceleration of pancreatic protein synthesis was found.[18] This is in sharp contrast to the findings in the threonine-devoid group, where there is an apparent inhibition of pancreatic protein synthesis.

The gastrocnemius muscle of rats force-fed the methionine-devoid diet showed a significant decrease in weight, protein content, and nonprotein amino acid nitrogen content in comparison with the controls (Table 8). The magnitude of decrease is essentially the same as was found in the animals in the threonine-devoid group.

In immature rats fed the methionine-devoid and control diets ad libitum the liver protein and lipid were decreased in the experimental animals. The liver glycogen and pancreatic protein were similar in animals of both groups (Table 9).

In experiments in which adult rats were force-fed the methionine-devoid or complete diet for three or six days, there was a significant increase in liver lipid in female but not in male rats of the experimental group at both time intervals in comparison with the controls.[19] No highly significant differences were found in liver weight, protein, and glycogen and in pancreatic weight and protein in either sex of both groups (Table 11).

Comment

One of the interesting observations made during this study was the failure to find evidence of a general inhibition of protein synthesis in the livers of immature rats force-fed a diet devoid in threonine, despite the presence of obvious pathologic changes.[1] The total liver protein, the extent of incorporation of radioactive amino acids into liver and plasma protein, and, in one experiment, the increase in tryptophan peroxidase in response to injected tryptophan all showed no decrease in the experimental animals as compared to controls. In fact, the results of the radioactive experiments are suggestive of an increase in protein synthesis in the liver in the animals of the experimental group.

These results, which appear to be in conflict with many nutritional studies with ad libitum-fed diets deficient in essential amino acids,[20] are nevertheless in accord with those of Spector and Adamstone[21] with tryptophan-deficient and of Samuels and coworkers[15,22] with tryptophan-, isoleucine-, or phenylalanine-deficient diets force-fed to rats. Although Spector and Adamstone con-

cluded from their study that young rats lose liver protein when force-fed a tryptophan-deficient diet, they expressed their results in *concentration*, rather than *total amount per liver*. On recalculating their protein values in terms of total quantity per liver, it was found that their animals, like ours, showed no loss in total liver protein. The results of Samuels and collaborators are more obviously in accord with ours, since they also express their values in terms of total amount per liver.

It must be emphasized, however, that the failure to find a general inhibition of protein synthesis in the animals given the devoid diet does not rule out selective inhibition of the synthesis or turnover of one or more liver enzymes in these animals. In fact, Van Pilsum et al.[22] found that certain hepatic enzyme activities did decrease in animals force-fed diets devoid in some single essential amino acids.

The observed pattern of hepatic protein metabolism in force-fed rats is in obvious contrast to that obtained in animals fed the same threonine-devoid diet ad libitum. Under the latter condition, the liver loses considerable protein nitrogen, along with other cellular components. A similar difference between the results with force-fed and ad libitum regimens was found by Van Pilsum et al.,[22] using tryptophan-, isoleucine-, or phenylalanine-devoid diets.

The hepatic protein results in the force-fed group could possibly be explained by the large decrease in protein and free amino acid level in skeletal muscle, as determined in a single muscle, the gastrocnemius. The rapid loss of muscle protein could make amino acids available for protein synthesis in other organs, including the liver. The elevated nonprotein amino acid level in the liver is consistent with this possible explanation.

However, in our experiments, the animals fed the threonine-devoid diet ad libitum also showed a large decrease in muscle protein content. Yet, in these animals, the liver lost considerable protein, and no specific pathologic changes were found in many of the organs.[1] Therefore, the changes in skeletal muscle cannot by themselves explain the differences in the pattern of protein metabolism between the force-fed and the ad libitum-fed animals.

A comparison between the protein levels in liver and in skeletal muscle of animals force-fed and fed ad libitum the threonine-devoid and the methionine-devoid diets suggests that the skeletal muscle protein is a much more sensitive index of the nutritional state of the whole organism with regard to essential amino acids than is the liver protein. Apparently the skeletal muscle, unlike the liver, loses protein when rats are fed a threonine- or methionine-devoid diet, regardless of the quantity of the diet consumed. In human beings also, the skeletal muscle may be a more sensitive index of the nutritional state with regard to amino acids and protein, since Waterlow [23] found that muscle protein in children with kwashiorkor showed much greater decreases from the normal than did liver protein.

The changes in protein metabolism in the liver and skeletal muscle of the animals force-fed the threonine-devoid diet resemble those reported by Clark [24] and by Silber and Porter [25] in animals given cortisone or hydrocortisone (cortisol). These investigators found a decrease in muscle protein and nonprotein N and an increase in liver protein and nonprotein N in treated animals. This similarity between the patterns of protein metabolism suggested that some of the changes observed in our rats force-fed the threonine-devoid diet might be due to increased cortisone production by the adrenal gland. The large increase in liver glycogen in these animals is consistent with this explanation.[26] However, the following observations do not support this suggestion: (*a*) rats given cortisone injections rapidly lost a large amount of their body weight, in contrast to the slight loss of weight in the animals of the threonine-devoid group, and (*b*) adrenalectomized rats given injec-

tions of hydrocortisone reacted to the force-feeding of single essential amino acid-devoid diets in the same manner as did intact rats, according to Van Pilsum and co-workers.[22] Thus an intact adrenal gland, capable of reacting by increased output of steroids, is apparently not essential for the animal's response to the devoid diets.

Since most of our animals were young, in a period of rapid growth, it was considered desirable to compare the number of cells in the livers of the animals of the experimental and control groups. Since nuclei have been shown to contain a constant amount of DNA in the somatic cells of any one species,[17] their relative number in any organ, in the absence of changes in ploidy, can be determined by measurement of the DNA content. Our results failed to show any difference in the DNA content between the livers of the experimental and the control animals. Since no obvious differences in the number of nuclei per liver cell were observed between the animals of the two groups,[1] we must conclude that the livers in each group had the same number of cells. In contrast to the results with DNA, the RNA level was significantly increased in the livers of the animals force-fed the threonine-devoid diet. This observation may be related to the increased incorporation of radioactive amino acids, since there is increasing evidence for a special role of nucleic acids in protein formation.[27-29]

One of the main purposes of this study was to determine whether a general inhibition of hepatic protein synthesis would occur prior to the development of a periportal fatty liver, as was observed with ethionine administration.[30] The present investigation has failed to uncover such a relationship when the fatty liver was produced by the force-feeding of diets devoid in either threonine or methionine. This raises the possibility that the pathogenesis of periportal fatty livers induced by various experimental conditions is different. No conclusion on this point is possible at this time, although certain observations are consistent with this suggestion. For example, with the threonine-devoid diet, the increment in liver lipid is composed of phospholipid and cholesterol as well as neutral fat. In contrast, with ethionine[31] the increment is all neutral fat. Also, with ethionine[32,33] and with the methionine-devoid diet[19] in mature rats, the females develop a fatty liver but the males do not. With the threonine-devoid diet, on the other hand, no sex difference has been observed.[19]

Samuels et al.[15] suggested that the fatty liver observed in their animals when they were force-fed tryptophan-, isoleucine-, or phenylalanine-devoid diets was due to increased synthesis of liver lipid from the carbon chains of amino acids which were not being used normally for protein synthesis. The findings in our animals given threonine- or methionine-devoid diets do not support this suggestion. With use of C^{14}-labeled leucine, isoleucine, or valine, it was found that the radioactivity in the liver lipids from the experimental rats was not significantly different from that in the controls. The pathogenesis of the fatty liver must therefore be sought in a disturbance of some other metabolic pathway. Conceivably, an interference with the synthesis or activity of one or more enzymes concerned with the handling of lipid by liver cells might be involved.

The biochemical data in the pancreas are consistent with the morphologic observations described in the previous paper.[1] In the animals of the threonine-devoid group, in which pancreatic edema with acinar atrophy was observed,[1] there were suggestive but not conclusive indications of a decreased ability of the pancreas to synthesize protein. In contrast, with methionine-devoid diets, no gross or microscopic evidence of edema or atrophy were found and no evidence for decreased protein synthesis in the pancreas of these animals was obtained. In fact, the results with the methionine-devoid diet were highly suggestive of increased protein synthesis in

animals force-fed this diet. This tentative conclusion is in agreement with results obtained in previous studies on ethionine.[18] Ethionine administration was found to produce an increase in enzyme and protein synthesis in the pancreas of rats within 24 hours after its injection, an effect prevented by the simultaneous administration of methionine. The possibility that methionine or a metabolic derivative of methionine plays some role as a regulator of protein synthesis in the pancreas must be entertained.

In unreported experiments performed during this study, it was observed that rats responded to diets low in an essential amino acid in a manner similar to that already described for diets devoid of the amino acid. For example, when animals were force-fed and fed ad libitum diets containing only 25% of the recommended amount of threonine, the morphologic and biochemical changes closely resembled those seen with the devoid diet. However, the changes were less severe within the same time interval. Apparently, not only an absolute but also a partial deficiency of threonine may induce the syndrome described in this and the preceding paper.[1]

One of the important conclusions to be drawn from the results of this study concerns the relationship of the amount of a deficient diet consumed by an animal to the nature of the pathologic response. Judging from both the morphologic and the biochemical reactions in rats fed ad libitum and by stomach tube diets devoid of a single essential amino acid, radically different tissue reaction patterns may be produced by merely increasing or decreasing the intake (Table 12). For example, with a relatively high intake of a threonine-devoid diet, assured by force-feeding, preservation of liver protein, fatty liver, excessive liver glycogen, and obvious atrophy of many glandular organs was consistently produced, whereas with a smaller ad libitum intake of the same diet, there was loss of liver protein without obvious histopathologic changes in the liver or other organs. This dependence of the tissue response upon the quantity of diet consumed is not unique to diets deficient in amino acids but has also been observed with choline-deficient diets.[34] As pointed out in the previous communication,[1] a relatively high intake of carbohydrate is frequently coupled with inadequate amounts of protein in some human diets. Apparently human beings, particularly infants, may be induced by nonphysiological factors to consume relatively large quantities of deficient diets. This is in sharp contrast to experimental animals, such as the rat, which commonly show a drastic reduction in food intake when the diet is deficient in an essential component. It is therefore probable that some human deficiency conditions may be more closely simulated in animals by force-feeding than by ad libitum regimens.

TABLE 12.—*Comparison of Chemical and Morphologic Changes Produced in Immature Rats by Force-Feeding and by Ad Libitum Feeding of Threonine-Devoid Diets*

	Force-Feeding	Ad Libitum Feeding
Change in body weight	Slight decrease	Considerable decrease
Liver		
Weight	Increase	Decrease
Protein	No change	Decrease
Lipid	Increase (periportal)	Questionable decrease
Glycogen	Increase	Questionable decrease
Pancreas		
Weight	Increase	Increase
Protein	No change	Decrease
Amylase	Decrease	Decrease
Morphologic changes	Acinar atrophy & edema	No change
Skeletal muscle (gastrocnemius)		
Weight	Decrease	Decrease
Protein	Decrease	Decrease
Salivary glands		
Parotid	Atrophy	No change
Submaxillary	Atrophy	No change
Stomach		
Glandular portion	Atrophy & loss of mucin	No change
Adrenal		
Zona glomerulosa	Atrophy	No change

Probably the most important conclusion that may be made from this combined morphologic-biochemical study is that anatomic responses, closely resembling those reported to be characteristic of kwashiorkor, can be readily induced in rats by the force-feeding

of diets devoid in an essential amino acid. According to current concepts of amino acid deficiencies,[20] the pathogenesis of these morphologic changes could most easily be explained by an interference with cellular protein synthesis in the affected organs. However, the biochemical results in our study failed to show a general inhibition of protein synthesis in all organs studied and indicate that different organs respond metabolically to an amino acid deficiency in different ways. In the pancreas, protein synthesis was disturbed, and the available evidence is consistent with the thesis that such disturbances may be the basis for the production of acinar damage. However, in the liver, the failure to find any over-all disturbance in protein synthesis necessitates the search for other biochemical alterations which may be important in the pathogenesis of the liver lesions.

Summary

Selected studies on hepatic protein, lipid, and carbohydrate metabolism and on protein metabolism in the pancreas and skeletal muscle have been performed on rats force-fed or fed ad libitum purified diets devoid of threonine or methionine. The morphologic changes observed in these animals were reported in the previous communication.[1]

It was found that the animals force-fed a threonine-devoid diet showed no evidence of a general inhibition of protein synthesis in the liver, as measured by protein content, incorporation of radioactive amino acids into liver and plasma protein, and the response of the activity of tryptophan peroxidase to injected tryptophan. Animals tube-fed the devoid diet showed an increased liver neutral fat, cholesterol, and phospholipid and increased hepatic glycogen. The elevated liver lipid could not be accounted for by an accelerated conversion of the carbon chains of amino acids to lipid, as tested by administering three different labeled amino acids. The liver deoxyribonucleic acid content was the same in the experimental and the control animals, but the liver ribonucleic acid content was greater in the experimental animals. The pancreas in animals force-fed the threonine-devoid diet showed a suggestive decrease in protein synthesis. The right gastrocnemius muscle in these animals weighed less and contained considerably less protein than in control animals.

In animals force-fed a methionine-devoid diet, the only positive finding in the liver was an elevated liver lipid. The pancreas in these animals showed a suggestive increase in protein synthesis. The changes in the gastrocnemius muscle were similar in animals force-fed either methionine- or threonine-devoid diets.

The biochemical findings were very different, depending on whether the animals received the larger amounts of the threonine- and methionine-devoid diets given by force-feeding or the smaller amounts ingested ad libitum. In animals fed ad libitum both the liver and the gastrocnemius muscle showed large decreases in protein content and the liver did not show any elevation in lipid or glycogen.

These findings clearly demonstrate that the quantity ingested may be just as important as the composition of an amino acid-deficient diet in determining the morphologic and biochemical responses in rats.

The Dow Chemical Company, through Dr. W. Colby, supplied many of the amino acids used in this study. Some of the lysine and methionine was supplied by E. I. du Pont de Nemours and Company, through Dr. N. W. Flodin.

Tulane University School of Medicine, Department of Pathology, 1430 Tulane Ave. (12) (Dr. Farber).

REFERENCES

1. Sidransky, H., and Farber, E.: Chemical Pathology of Acute Amino Acid Deficiencies: I. Morphologic Changes in Immature Rats Fed Threonine-, Methionine-, or Histidine-Devoid Diets, A. M. A. Arch. Path., this issue, pp. 119.

2. Heller, A.; Plazin, J., and Van Slyke, D. D.: A Study of Conditions for Kjeldahl Determination of Nitrogen in Proteins, J. Biol. Chem. 176: 1401-1420, 1948.

3. Carr, J. J., and Drekter, I. J.: Simplified Rapid Technic for the Extraction and Determination of Serum Cholesterol Without Saponification, Clin. Chem. 2:353-368, 1956.

4. Fiske, C. H., and Subbarow, Y.: Colormetric Determination of Phosphorus, J. Biol. Chem. 66: 375-400, 1925.

5. Cori, G. T., and Cori, C. F.: Glucose-6-Phosphatase of the Liver in Glycogen Storage Disease, J. Biol. Chem. 199:661-667, 1952.

6. Knox, W. E., and Auerbach, V. H.: The Hormonal Control of Tryptophan Peroxidase in the Rat, J. Biol. Chem. 214:307-313, 1955.

7. Smith, B. W., and Roe, J. H.: A Photometric Method for the Determination of α-Amylase in Blood and Urine, with Use of the Starch-Iodine Color, J. Biol. Chem. 179:53-59, 1949.

8. Farber, E., and Sidransky, H.: Changes in Protein Metabolism in the Rat Pancreas on Stimulation, J. Biol. Chem. 222:237-248, 1956.

9. Seifter, S.; Dayton, S.; Novic, B., and Muntwyler, E.: The Estimation of Glycogen with the Anthrone Reagent, Arch. Biochem. 25:191-200, 1950.

10. Carroll, N. V.; Longley, R. W., and Roe, J. H.: The Determination of Glycogen in Liver and Muscle by Use of Anthrone Reagent, J. Biol. Chem. 220:583-593, 1956.

11. Frame, E. G.; Russel, J. A., and Wilhelmi, A. E.: The Colorimetric Estimation of Amino Nitrogen in Blood, J. Biol. Chem. 149:225-270, 1943.

12. Russel, J. A.: Note on the Colorimetric Determination of Amino Nitrogen, J. Biol. Chem. 156:467-468, 1944.

13. Schmidt, G., and Thannhauser, S. J.: A Method for the Determination of Desoxyribonucleic Acid, Ribonucleic Acid, and Phosphoproteins in Animal Tissues, J. Biol. Chem. 161:83-89, 1946.

14. Logan, J. E.; Mannell, W. A., and Rossiter, R. J.: Estimation of Nucleic Acids in Tissue from the Nervous System, Biochem. J. 51:470-482, 1952.

15. Samuels, L. T.; Goldthorpe, H. C., and Dougherty, T. F.: Metabolic Effects of Specific Amino Acid Deficiencies, Fed. Proc. 10:393, 1951.

16. Cori, G. T.: Glycogen Structure and Enzyme Deficiencies in Glycogen Storage Disease, Harvey Lect. 48:148-171, 1952-1953.

17. Vendrely, R., and Vendrely, C.: La Teneur du noyau cellulaire en acide désoxyribonucléique à travers les organes, les individus et les espèces animales, Experientia 4:434-436, 1948; 5:327-329, 1949.

18. Sidransky, H., and Farber, E.: The Effects of Ethionine upon Protein Metabolism in the Pancreas of Rats, J. Biol. Chem. 219:231-243, 1956.

19. Sidransky, H., and Farber, E.: Sex Difference in the Induction of Periportal Fatty Liver by Methionine Deficiency in the Rat, Proc. Soc. Exper. Biol. & Med., to be published.

20. Cannon, P. R.: Some Pathologic Consequences of Protein and Amino Acid Deficiencies, Springfield, Ill., Charles C Thomas, Publisher, 1948.

21. Spector, H., and Adamstone, F. B.: Tryptophan Deficiency in the Rat Induced by Forced Feeding of an Acid Hydrolyzed Casein Diet, J. Nutrition 40:213-229, 1950.

22. Van Pilsum, J. F.; Speyer, J. F., and Samuels, L. T.: Essential Amino Acid Deficiency and Enzyme Activity, Arch. Biochem. 68:42-53, 1957.

23. Waterlow, J. C.: The Protein Content of Liver and Muscle as a Measure of Protein Deficiency in Human Subjects, West Indian M. J. 5:167-174, 1956.

24. Clark, I.: The Effect of Cortisone upon Protein Synthesis, J. Biol. Chem. 200:69-76, 1953.

25. Silber, R. H., and Porter, C. C.: Nitrogen Balance, Liver Protein Repletion and Body Composition of Cortisone Treated Rats, Endocrinology 52:518-525, 1953.

26. Ingle, D. J.: The Biologic Properties of Cortisone: A Review, J. Clin. Endocrinol. 10:1312-1354, 1950.

27. Caspersson, T.: Cell Growth and Cell Function: A Cytochemical Study, New York, W. W. Norton & Company, Inc., 1950.

28. Brachet, J.: The Localization and the Role of Ribonucleic Acid in the Cell, Ann. New York Acad. Sc. 50:861-869, 1950.

29. Gale, E. F., and Folkes, J. P.: Effect of Nucleic Acids on Protein Synthesis and Amino-Acid Incorporation in Disrupted Staphylococcal Cells, Nature, London 173:1223-1227, 1954.

30. Farber, E.: Relationship of Disturbed Hepatic Protein Metabolism to Fatty Liver Induced by Ethionine, Fed. Proc. 14:402, 1955.

31. Jensen, D.; Chaikoff, I. L., and Tarver, H.: The Ethionine-Induced Fatty Liver: Dosage, Prevention, and Structural Specificity, J. Biol. Chem. 192:395-403, 1951.

32. Farber, E.; Simpson, M. V., and Tarver, H.: Studies on Ethionine: II. The Interference with Lipide Metabolism, J. Biol. Chem. 182:91-99, 1950.

33. Farber, E.; Koch-Weser, D., and Popper, H.: The Influence of Sex and of Testosterone upon Fatty Liver Due to Ethionine, Endocrinology, 48:205-212, 1951.

34. Best, C. H.: Protection of Liver and Kidneys by Dietary Factors: Choline and Its Precursors as Lipotropic Agents, Fed. Proc. 9:506-511, 1950.

The Feulgen Reaction of Fowlpox Inclusions After Lipid Extraction

WILLIAM M. TODD, Ph.D., and CHARLES C. RANDALL, M.D., Jackson, Miss.

The nature of the inclusion body produced during fowlpox infection has been the subject of a number of papers over a period of many years. Sanfelice,[1] Michaelis,[2] Burnet,[3] and Ludford and Findlay[4] have studied the inclusion microscopically, employing hematoxylin-and-eosin-stained preparations and preparations stained for lipids and for specific cellular structures. Others[5-7] have recently investigated the virus, using electron microscopy. The relationship between the inclusion body and the Feulgen reaction has been briefly noted by Findlay,[8] who has suggested that the faintly positive reaction which he observed might be due to the inclusion matrix which surrounds the elementary bodies. The above authors have shown that fowlpox inclusions readily take up osmic acid and other lipid stains, and it is also well known that certain lipids, namely, plasmalogens, may give rise to false-positive Feulgen reactions.

In this laboratory a systematic study of fowlpox-infected tissue is in progress, and it was therefore decided to investigate more fully the fowlpox inclusion body during its developmental cycle with use of the Feulgen technique and to determine whether or not the presence of lipid in this inclusion body might lead to an erroneous interpretation.

Materials and Methods

Virus.—The strain of fowlpox virus used in this study was supplied by Dr. E. R. Doll,* in 1950, having been isolated from a field case of the disease. Prior to this study it had been passaged four times in chick scalp. Stock virus for various inoculations was obtained by another passage in a large number of chicks. After seven days the skin lesions were harvested, triturated, and diluted to 1:5 or 1:10 with isotonic saline containing skim milk (10% volume per volume), and the virus suspension was frozen and maintained at —20 C. Before inoculation the stock virus suspension was diluted to 1:20 with saline and applied to plucked chick scalps.

Animals.—Day-old "Hyline" Cockerels obtained from a local commercial hatchery were employed as the experimental host.

Histologic Methods.—One or more chicks were killed at spaced intervals after inoculation, and representative specimens of scalp were placed in one or more of the following fixatives: Zenker, Zenker-formol, 10% formaline, formol-acetic, or Carnoy's solution (6:3:1). Fixation times varied with the fixative and with the size of the block of tissue. Sections for microscopic examination were cut uniformly at 6μ in thickness. Hematoxylin-and-eosin stains were made on all specimens of tissue. Feulgen nuclear reactions were performed by the modified method of Feulgen and Rossenbeck,[9] employing Schiff's reagent as prepared by de Tomasi.[10] Optimum hydrolysis times for specimens fixed in formalin, formol-acetic, Zenker-formol, and Carnoy's solution were determined to be 7, 11, 7, and 8 minutes, respectively. All slides remained in Schiff's reagent for two hours.

Sections were also prepared from blocks of infected tissue which had been extracted for 30 minutes in boiling absolute ethanol followed by 30 minutes' extraction in boiling Bloor's solvent followed by 24 hours' extraction in a Soxhlet apparatus with diethyl ether. As a check of the extraction procedure, the tissues were further extracted for 24 hours with fresh diethyl ether. One-third (50 ml.) of the ether was evaporated to dryness in a weighing bottle, and the bottle was reweighed. There was no lipid residue to 0.1 mg. Sections from these tissues were stained with hematoxylin and eosin, and the Feulgen and plasmal reactions were performed on representative sections both with

Submitted for publication Feb. 13, 1958.

This investigation was aided by U. S. Public Health Service Grant E-1083.

Department of Microbiology, University of Mississippi School of Medicine.

* Department of Animal Pathology, Kentucky Agriculture Experiment Station, University of Kentucky, Lexington, Ky.

and without prior hydrolysis. Control sections of the unextracted tissues were stained without prior hydrolysis to test for false-positive reactions. Sections were also briefly exposed to mercuric chloride to test for the plasmal reaction. Other controls, which were stained with and without previous hydrolysis, consisted of sections of tissue of known deoxyribonucleic acid concentration,[11] namely, 10.7×10^{-9} mg. per nucleus.

Results

Microscopic observation of hematoxylin-and-eosin-stained sections agreed with those of previous investigators as to the development of the lesions and inclusion bodies. No essential differences in staining or in results were noted among various fixatives.

Foreign-Stained Sections.—A few ill-defined Feulgen-negative inclusion bodies were noted in infected cells 48 hours after inoculation. The nuclei of these cells appeared to be enlarged and presented coarsely divided and sometimes marginated chromatin. The nucleoli were swollen and appeared as darkly staining rings. It was noted that as inclusion bodies developed within the cytoplasm of infected cells, the nuclei stained less intensely with Schiff's reagent than nuclei of uninfected cells. Until 72 hours the Feulgen reaction of the inclusion bodies was equivocal. After this time they became definitely positive and continued to give a positive reaction as long as the study continued (12 more days).

All unhydrolyzed control sections were Feulgen-negative, showing a complete absence of color. The plasmal reaction was negative both in lipid-extracted tissues and in those fixed in the usual manner.

In sections made from tissues which had been fixed by the usual methods, the large eosinophilic intracytoplasmic inclusions had a glistening appearance, in many instances resembling a mass of lipid material (Fig. 1). Elementary bodies as such could not be recognized. All sections made from the lipid-and-water-extracted tissue showed some loss of architecture. This consisted of general shrinkage, probably due to water loss, and of partial loss of the cornified epithelial layer of cells. In both hematoxylin-and-eosin- and in Feulgen-stained sections, the inclusions were variable in appearance. Some were dull and faintly granular, and the matrix appeared intact (Fig. 2). In others the matrix was apparently extracted, and fine particles, consistent in size with fowlpox elementary bodies, were dispersed throughout the cells (Fig. 3). The Feulgen reaction of extracted inclusions was questionably less intense than those in unextracted tissue. In the instance where the elementary bodies were dispersed throughout the cell so that they could be individually examined, they appeared weakly positive.

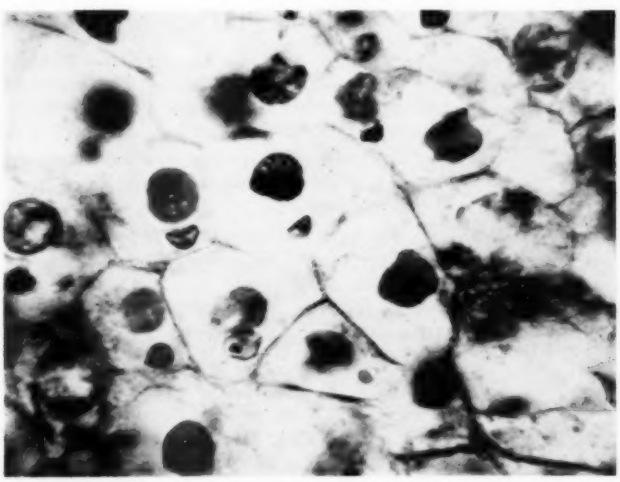


Fig. 1.—Photomicrograph of chick scalp infected with fowlpox for seven days. The large dense intracytoplasmic inclusions are conspicious in the epithelial cells. Hematoxylin and eosin, formalin fixed; × 945.

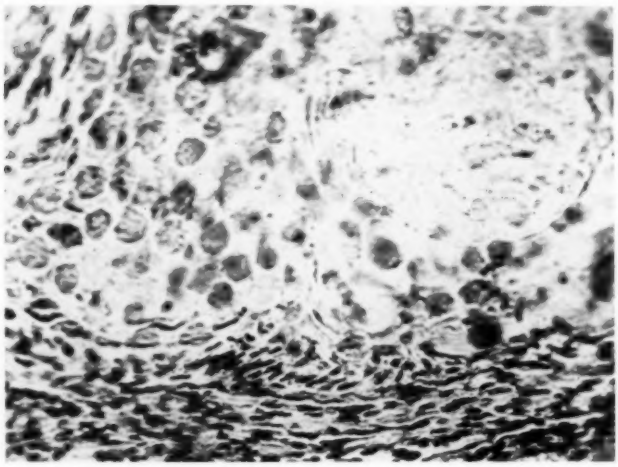


Fig. 2.—Photomicrograph of feather follicle of same tissue as Figure 1. Feulgen stain after water and lipid extraction. The epithelial cells contain granular, distinct, intracytoplasmic Feulgen-positive inclusions. High power, formalin fixed; × 472.

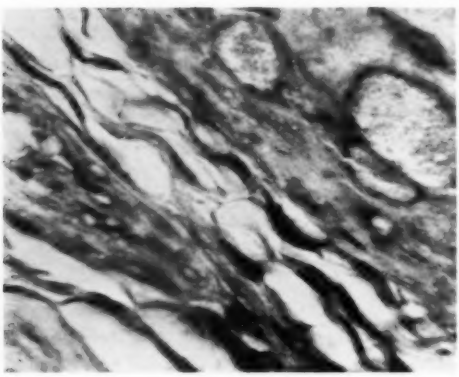


Fig. 3.—Photomicrograph of same tissue as Figure 2, through edge of infected feather follicle. The inclusions have been broken in the process of extraction and the elementary bodies dispersed throughout the cell. Hematoxylin and eosin, formalin fixed; × 945.

Further work is in progress on isolated inclusions to determine the chemical composition of both the matrix and the elementary bodies.

Summary

The presence of Feulgen-positive material in the cytoplasmic inclusion bodies occurring in chick cells infected with fowlpox virus is established. It is shown by various controls that this is not a false-positive reaction and that the reaction is not due to the presence of lipid in the inclusion matrix.

University of Mississippi, Medical Center, Department of Microbiology.

REFERENCES

1. Sanfelice, F.: Über die pathogene Wirkung der Blastomyceten: IV. Beiträge sur Aetiologie der sog. Pocken der Tauben (Geflügelpocken), Ztschr. Hyg. 26:298-322, 1897.

2. Michaelis, L.: Mikroskopische Untersuchungen über die Taubenpocke, Ztschr. Krebsforsch. 1:105-111, 1904.

3. Burnet, E.: Contribution à l'étude de l'épithélioma contagieux des oiseaux, Ann. Inst. Pasteur 20:742-765, 1906.

4. Ludford, R. J., and Findlay, G. M.: The Ultra-Microscopic Viruses: II. The Cytology of Fowl-Pox, Brit. J. Exper. Path. 7:256-264, 1926.

5. Bang, F. B.; Levy, E., and Gey, G. O.: Some Observations on Host-Cell-Virus Relationships in Fowl Pox: I. Growth in Tissue Culture; II. The Inclusion Produced by the Virus on the

Chick Chorio-Allantoic Membrane, J. Immunol. 66:329-345, 1951.

6. Morgan, C.; Ellison, S. A.; Rose, H. M., and Moore, D. H.: Structure and Development of Viruses Observed in the Electron Microscope: II. Vaccinia and Fowl Pox Viruses, J. Exper. Med. 100:301-310, 1954.

7. Eaves, G., and Flewett, T. H.: The Structure of Fowl-Pox Inclusions (Bollinger Bodies), J. Hyg. 53:102-105, 1955.

8. Findlay, G. M., in Handbuch der Virusforschung, Vienna, Springer-Verlag, 1938, Vol. 1.

9. Feulgen, R., and Rossenbeck, H.: Mikroskopisch-chemischer Nachweis einer Nucleinsäure vom Typus der Thymonucleinsäure und die darauf beruhende elective Färbung von Zellkernen in mikroskopischen Präparaten, Ztschr. physiol. Chem. 135:203-248, 1924.

10. de Tomasi, J. A.: Improving the Technic of the Feulgen Stain, Stain Technol. 11:137-144, 1936.

11. Bracken, E. C., and Randall, C. C.: Studies on Hepatitis in Hamsters Infected with Equine Abortion Virus: II. Changes in Protein, Nucleic Acid, and Weight of Isolated Nuclei, Am. J. Path. 33:1009-1022, 1957.

Experimental Production of Peritoneal Lipogranulomas in the Rat

JOHN A. EISENSCHMID, A.B., and J. L. ORBISON, M.D., Rochester, N. Y.

There are numerous reports in the recent literature of lipoid pneumonias and subcutaneous lipogranulomas due to liquid petrolatum (mineral oil). There have recently been several cases reported by Norris and Davidson,[1] Whitaker et al.,[2] Ruffin,[3] and Cruickshank,[4] of peritoneal lipogranuloma following the introduction of liquid petrolatum into the peritoneal cavity at the time of surgery. This latter disease is characterized by massive adhesions within the peritoneal cavity, numerous small lipogranulomas covering the peritoneal surfaces, and large calcified oil-containing cysts which have a characteristic appearance on x-ray examination.

During the first two decades of this century numerous authors advocated using a great number of substances to prevent peritoneal adhesions, including hydrous wool fat (lanolin), collodion, petrolatum, liquid petrolatum, and olive oil and other vegetable oils. Wilkie,[5] Gellhorn,[6] and Blake[7] carried out some short-term animal experiments with these substances and believed that liquid petrolatum was especially beneficial in preventing postoperative peritoneal adhesions. Cubbins and Abt[8] and Pope[9] later stated that these substances had little effect on preventing postoperative adhesions and that, in fact, they might be intensely irritating to the peritoneal surfaces.

Recently, Squire et al.[12] have noted several cases of peritoneal lipogranuloma at this hospital. In each instance characteristic x-ray and pathological features were found. Since all of the animal experiments dealing with mineral oil in the peritoneal cavity have been of short duration except for Tantini's[10] report, in 1935, it was decided to study the long-term effects.

Submitted for publication March 11, 1958.

The Department of Pathology, University of Rochester School of Medicine and Dentistry.

Method

Thirty rats of the Wistar strain given a stock diet of Purina Dog Chow were each given an intraperitoneal injection of 5 cc. of sterile (autoclaved for 10 minutes at 15 lb. at 121° C) liquid petrolatum after an iodine-alcohol preparation of the abdominal wall. Of these 30 animals 6 died spontaneously. Four of these animals were completely autopsied and were found to have died from empyema and severe bronchopneumonia. The other two rats were not autopsied owing to severe maceration and were omitted from the study.

Animals were killed in groups of three to five at intervals of 2, 5, 10, 21, and 32 weeks, and animals that died spontaneously, mostly of pneumonia or bronchiectasis, were observed at 3 days and at 7, 20, and 26 weeks. Complete autopsies were done on all killed animals, and routine hematoxylin-and-eosin-stained histological sections were made. Special stains, including periodic acid-Schiff and calcium stains (Becker method), were done where indicated.

Results

Twenty-seven of the twenty-eight animals studied showed reactions to intraperitoneal liquid petrolatum. One animal killed at 21 weeks showed no gross or microscopic lesions within the peritoneal cavity. It is believed that this lack of reaction may have been due to perforation of the bowel at the time of injection, with the oil being injected into the lumen of the bowel.

The initial reaction noted in one animal at three days was one of acute peritonitis, with fibrin covering portions of the peritoneal surfaces. Emulsified liquid petrola-

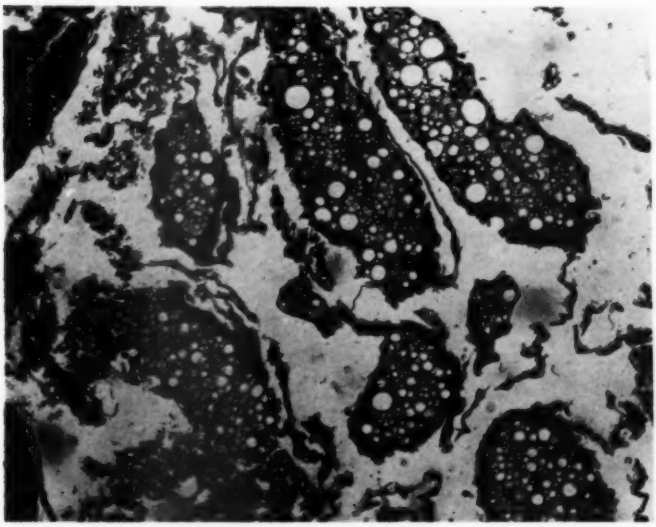


Fig. 1.—Five weeks after injection of liquid petrolatum. Section through mesentery, showing nodules composed of numerous small vacuoles with slight amounts of fibrous tissue surrounding the periphery of the nodules. Hematoxylin and eosin; reduced about 15% from mag. × 100.

tum was seen free within the peritoneal cavity. Microscopically there was a reaction consisting of fibrin and polymorphonuclear cells covering portions of the peritoneal surfaces, with no signs of organization.

Animals killed at two weeks had a thin layer of oily-appearing substance covering the peritoneal surfaces and numerous small grayish white nodular lesions, many of which were freely movable, covering the surfaces of the omentum and mesentery. Microscopically, these lesions appeared as collections of macrophages containing vacuoles. In the central portion of these lesions the cells appeared to have undergone degeneration, leaving large cyst-like areas filled with what is believed to be liquid petrolatum. At the periphery of these lesions fibroblastic proliferation had occurred but very little collagen production. There were also small foci of lymphocytes and a few poly-

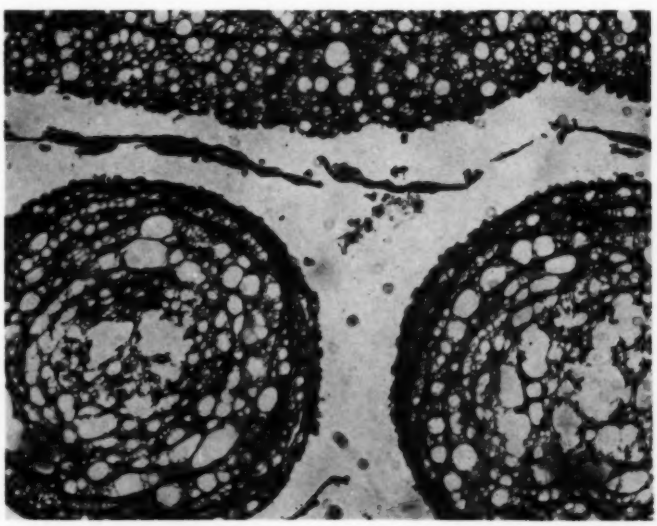


Fig. 2.—Ten weeks after liquid petrolatum injection. Section showing two nodules composed of numerous cysts with fibrous septa. Note the break-down of the septa in the central areas of the nodules. Hematoxylin and eosin; reduced about 15% from mag. × 100.

morphonuclear leukocytes within these granulomatous regions.

At five weeks there were only traces of free oil within the peritoneal cavity and an increased number of granulomas compared to the findings in the animals killed at two weeks. The lesions now varied from 0.5 to 3 mm. in diameter. Although some were still freely movable, most were bound to the peritoneal surface. There were marked accumulations of these lesions in the subdiaphragmatic region, between the lobes of the liver, and at the base of the mesentery. Microscopically, the only change from the picture at two weeks was a slight increase in the amount of fibrous tissue surrounding both the small granulomas and the individual cysts. The cysts in general seemed to be of a larger size, and there were a few large multinucleated foreign-body giant cells interspersed between the fibrous walled cysts.

Ten weeks after injection of liquid petrolatum, the reaction included a few large plaques, composed of aggregates of small cyst-like structures. These covered part of the surface of the liver, especially in the subdiaphragmatic region. A few fine adhesions between folds of omentum were noted both grossly and microscopically. Microscopically, several large nodules showed extensive central necrosis and one contained small amounts of calcium.

Animals killed at 21 and 32 weeks showed mainly quantitative changes from the above groups, with a marked increase in the size of the individual granulomas and a greater number of large plaques composed of aggregates of small cysts. Delicate fibrous adhesions were also present in greater numbers in these two groups.

One animal killed at 21 weeks and one, at 32 weeks showed large masses composed of matted omentum and adherent bowel. On sectioning, these masses were grayish yellow, slightly oily, and firm. On microscopic examination these two masses contained several large necrotic areas in which only outlines of cyst walls remained. There were also small foci of calcification present. The margins of these masses were composed of large amounts of dense collagenous tissue and considerable fibroblastic proliferation.

Sections of the spleens of all animals contained no lipid accumulations or lipid-containing macrophages. Two lymph nodes were noted to contain numerous eosinophilic, periodic acid-Schiff-positive Russell bodies,

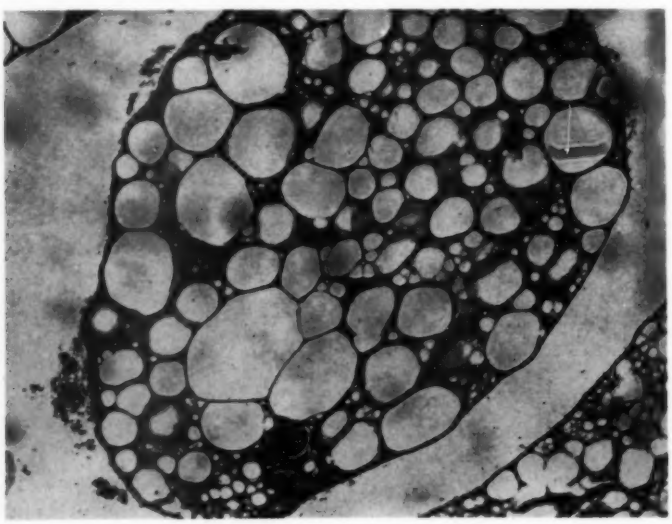


Fig. 3.—Twenty-one weeks after injection of liquid petrolatum. This section demonstrates the progressive enlargement of the granulomatous nodules and increased size of the individual cysts and thickening of the septa. Hematoxylin and eosin; reduced about 15% from mag. × 100.

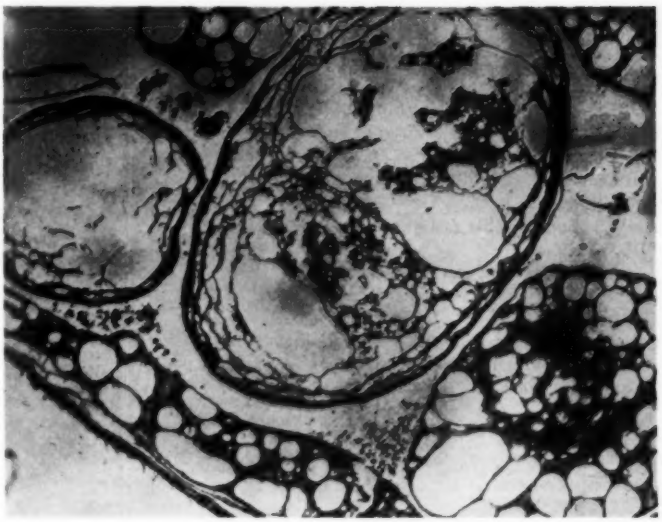


Fig. 4.—Twenty-one weeks after injection of liquid petrolatum. There are several large cysts with thick fibrous walls and areas of hyalinization. Within the central area of the central cyst is a granular deposit of calcium. Hematoxylin and eosin; reduced about 15% from mag. × 30.

but further search for these structures was of no avail.

Comment

From the above studies it is apparent that liquid petrolatum placed in the peritoneal cavity of rats induces a foreign-body type inflammatory reaction. The characteristic response consists of adhesions between loops of bowel and mesentery and oil-filled fibrous-walled cysts, with lymphocytes and multinucleated giant cells interspersed between. Calcification also occurred in several of the cysts.

The above observations agree essentially with those of Tantini, who in 1935 showed that repeated intraperitoneal injections of liquid petrolatum over a period of about 18 weeks in guinea pigs induced free-floating and pedunculated granulomatous lesions. These nodules were composed of hyperplastic connective tissue with cystic areas containing liquid petrolatum.

Although the present findings did not include the dense matted adhesions or completely calcified oil-filled cysts seen in human peritoneal lipogranuloma, it is believed that the reaction is equivalent. The variations are probably explained by the difference in duration between the experimental and the human reactions, 32 weeks in the experimental animal and several years in most humans observed. Also, the fact that no surgery was performed on the animals prior to the instillation of liquid petrolatum may have played a role also in the quantitative difference in the reactions.

Stryker [11] showed that animals fed liquid petrolatum absorbed small amounts from the intestine and demonstrated characteristic lesions in mesenteric lymph nodes. Tantini noted fatty degeneration of the liver which was believed to be due to the liquid petrolatum. In the present study no lesions were present in spleen, lymph nodes, or liver, thus indicating that liquid petrolatum is not absorbed through an intact peritoneum.

Summary

The injection of liquid petrolatum (mineral oil) intraperitoneally into rats produces a progressive lipogranulomatous reaction similar to that observed in humans.

University of Rochester School of Medicine and Dentistry, 260 Crittendon Blvd. (20) (Dr. Orbison).

REFERENCES

1. Norris, J. C., and Davidson, T. C.: Peritoneal Reaction to Liquid Petrolatum, J. A. M. A. 103:1846, 1943.

2. Whitaker, W. G., Jr.; Walker, E. T., and Canipelli, J.: Lipogranuloma of the Peritoneum: Report of 3 Cases Following Intra-Abdominal Use of Liquid Petrolatum, J. A. M. A. 138:363, 1948.

3. Ruffin, J.: Oil Granuloma of the Peritoneum, Gastroenterology 20:485, 1952.

4. Cruickshank, A. H.: Paraffinoma of the Peritoneum, Lancet 1:4, 1941.

5. Wilkie, D. P. E.: The Use of Oil in Abdominal Surgery, Surg. Gynec. & Obst. 10:126, 1910.

6. Gellhorn, G.: Experimental Studies of Post-Operative Peritoneal Adhesions, Surg. Gynec. & Obst. 8:505, 1909.

7. Blake, J. B.: The Use of Sterile Oil to Prevent Peritoneal Adhesions, Surg. Gynec. & Obst. 6:667, 1908.

8. Cubbins, W. R., and Abt, J. A.: A Preliminary Report Concerning the Effect of Foreign Substances in the Peritoneal Cavity, Surg. Gynec. & Obst. 22:571, 1916.

9. Pope, S.: The Prevention of Peritoneal Adhesions by the Use of Citrate Solutions, Ann. Surg. 63:205, 1916.

10. Tantini, E.: Processi produttivi da olio di paraffina in peritoneo nella cavia, Arch. Ist. biochim. ital. 7:27, 1935.

11. Stryker, W. A.: Absorption of Liquid Petrolatum ("Mineral Oil") from the Intestine: Histologic and Chemical Study, Arch. Path. 31: 670, 1941.

12. Squire, L. F.; Levine, I.; Eisenschmid, J. A., and Pearse, H. E.: To be published.

Unusual Testicular Histology in "True" Klinefelter's Syndrome

J. R. AUGUSTINE, M.D., and Z. F. JAWORSKI, M.D., Toronto

In 1942, Klinefelter and associates [1] described a syndrome characterized by gynecomastia, small testes with azoospermia, a varying degree of eunuchoidism, and elevated urinary gonadotropins. On biopsy the testes showed atrophy of the seminiferous tubules but normal interstitial cells. In a later review of the subject, Nelson and Heller [2] stressed that the only constant feature of the condition was the testicular histology.

The genetic sex of these patients was not questioned until a clinical test for genetic sex was developed from the original observation of Barr and Bertram [3] that female cells contained an aggregation of nuclear chromatin not found in male cells. This test, using either a skin biopsy specimen or a smear of buccal mucosa, has been applied by many workers to the study of human intersexes and related conditions.[4-11] In this manner, some of the cases of Klinefelter's syndrome were found to be genetic females. Nelson [4] designated these patients with chromatin-positive nuclei as cases of "true" Klinefelter's syndrome. He refers to the genetic males with similar clinical features as cases of "false" Klinefelter's syndrome.

In Nelson's view, "true" Klinefelter's syndrome represents an almost complete sex reversal. The pathogenesis is not clear, but most authors [5,6] agree that some adverse influence acts on the undifferentiated gonad of the genetically female embryo to interfere with the development of its female elements. The gonad then becomes an imperfect testis, which is still able to govern development of the genital tract along male lines.

In the pathogenesis of "false" Klinefelter's syndrome, a variable degree of development of the testis is achieved before damage occurs. Therefore, the seminiferous tubules in these cases are able to attain a larger size than the tubules found in "true" Klinefelter's syndrome. The other differences in the testicular histology and clinical features are set out in the Table and are illustrated by the accompanying photomicrographs.

In this paper is reported the case of a patient with "true" Klinefelter's syndrome. The postmortem examination showed an almost complete absence of interstitial cells in the testis. The patient also had alcoholic cirrhosis, which may account for this unusual testicular histology.

History and Findings

The patient, aged 52, was admitted to the surgical service of the Toronto General Hospital on June 28, 1957, because of ulcers of the left lower leg. This was the last of a series of admissions initiated by a comminuted fracture of the lower end of the left tibia following a fall in September, 1953. The fracture was treated by closed reduction and lengthy immobilization, but union did not occur. In 1955, a septic arthritis developed in the involved joint. In February, 1957, an arthrodesis was attempted, but no union resulted. The left leg was kept in a cast until a month prior to the last admission, when, because of the discomfort and irritation, the cast was removed. A peculiar red velvety nodular eruption progressing to ulceration developed in this area.

Examination.—On admission his temperature was 101 F, and he was orthopneic and appeared acutely

Submitted for publication Feb. 25, 1958.

From the Departments of Pathology and Medicine, The University of Toronto. Graduate Medical Research Fellow, National Research Council of Canada (Dr. Augustine); Arthur Haasz Fellow in Medicine (Dr. Jaworski).

Differentiating Points in "True" and "False" Klinefelter's Syndrome

	Klinefelter's Syndrome	
	"True"	"False"
Genetic sex	Usually female chromatin pattern (XX)	Male chromatin pattern (XY)
Onset of lesion	Prior to, or at time of, gonadal differentiation	After gonadal differentiation
Clinical picture	Small testes; gynecomastia frequent; masculinization usually incomplete, and patient may be eunuchoid	Small testes; gynecomastia infrequent; masculinization usually complete
Laboratory findings	Urinary gonadotropins usually increased; urinary 17-ketosteroids normal or low	
Histology of testes	Tubules—small, immature, and faintly hyalinized throughout the testicle Spermatogenesis—very rare. Interstitial cells—in clumps about vessels (see Fig. 3)	Tubules—normal in size, usually lined by Sertoli cells, and hyalinized in focal areas Spermatogenesis—may occur but is usually deficient Interstitial cells—diffuse throughout the testicle (see Fig. 4)

ill. There was jugular venous distention, a left pleural effusion, and rales in both lung bases. The cardiac rate was 120 per minute and regular; no murmurs were heard. The blood pressure was 170/90. The liver was enlarged to 3 fingerbreadths below the costal margin. Pitting edema of both ankles was present.

He weighed 270 lb. and was 6 ft. 6 in. tall. His extremities were thin and appeared longer than normal, but no measurements were taken. His voice was high pitched, and his skin was soft and delicate. He had no facial or axillary hair, and only scant pubic hair was present. His breasts were more prominent than could be accounted for by his truncan obesity. The penis was about 2 in. long. The testes were felt in the scrotum and were about a quarter of the normal size.

Laboratory Investigation.—Urinalysis was normal; Hb., 12.5 gm.; sedimentation rate, 70 mm. in one hour; W. B. C., 6000, and platelet count, 150,000. Blood Wassermann test was strongly positive. Skin biopsy performed on July 19, 1957, from the nodules on the left lower leg showed a malignant lymphoma.

Treatment and Course.—A provisional diagnosis of systemic lymphoma was made. The patient was treated with triethylene melamine and prednisone. A few days prior to death jaundice appeared. After two days in coma he died, on Sept. 1, 1957.

Special Investigation.—Shortly before he died, an oral buccal smear had been taken to determine his genetic sex. This was reported by Prof. Murray Barr, of the University of Western Ontario, as showing female sex chromatin pattern.

Personal History and Past Illnesses.—The patient completed Grade 7 and since then had worked at various unskilled occupations. He married in 1927, when 22 years of age, and was separated in 1939 because of his wife's presumed promiscuity. Early in their marriage, his wife bore a child who died of pneumonia at 9 months of age. Another pregnancy terminated in stillborn twins. The sex of none of these children could be established.

After a head injury in 1929 he became very obese and reported that his beard stopped growing. His greatest recorded weight was 390 lb., and his average weight was 340 lb. He was reported to have been a heavy drinker most of his life.

In 1941, a routine serological test for syphilis was found to be strongly positive. The spinal fluid was negative. He received a full course of oxophenarsine hydrochloride (Mapharsen) and bismuth in 1943 and penicillin in 1952.

Necropsy Findings

Anatomical Diagnosis

1. Aplasia of testes. 2. Gynecomastia. 3. Obesity. 4. Reticulum-cell sarcoma in lungs, pleura, peritoneum, pancreas, right adrenal gland, ileum, colon, skin of left lower leg, and retroperitoneal lymph nodes. 5. Portal cirrhosis (alcoholic). 6. Splenomegaly. 7. Hepatocellular jaundice. 8. Ascites (3000 cc.). 9. Left pleural effusion (2000 cc.). 10. Bilateral pleural adhesions. 11. Old ununited fracture of the left tibia. 12. Regional atrophy of the thyroid gland. 13. Hypoplasia of the prostate. 14. Excessive erythrophagocytosis of spleen and marrow. 15. Hypertrophy of the kidneys (histologically normal).

Comment

Three features of the necropsy deserve comment. The first was the widely disseminated malignant lymphoma with the histological characteristics of a reticulum-cell sarcoma. The pathological details are not pertinent to this report and are listed briefly in the summary. The spleen showed no evidence of tumor cells, and its weight of 640 gm. was explained by congestion and increase in reticulum cells, polymorphonuclear leukocytes, and erythrophagocytes.

The second feature was the cirrhosis. The liver weighed 2640 gm. and was nodular, and the cut surface appeared jaundiced. The gross picture was that of advanced cirrhosis, and this was confirmed by the microscopic sections. These showed progressive loss of liver cells and splitting

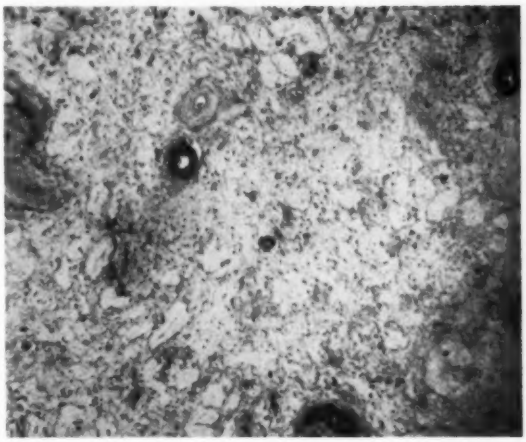


Fig. 1.—Low-power view of right testis in case reported. Note the absence of spermatogenesis, Sertoli cells, and interstitial cells. Hematoxylin and eosin; × 80.

of lobules by bands of condensed collagenous stroma. About half the liver cells showed fatty vacuolation. Cellular exudate, proliferation of bile ducts, and signs of growth of regeneration nodules were all very slight. The liver was typical, therefore, of a progressive cirrhosis, probably alcoholic in origin.

The third feature concerned the genital and endocrine systems. The penis and testes were infantile. The tubules of the epididymis were atrophied and distended by granular amorphous material, and no lipochrome pigment was present in the epithelium. The tubules of the rete testis were empty and lined by vacuolated cells, with very vesicular nuclei. The seminiferous tubules could be easily recognized only directly beneath the tunica albuginea. Here they had a thin external membrane and an inner broad convoluted cushion, taking very little stain of any kind. All these tubular outlines were small and prepubertal in size (Figs. 1 and 2). In the center of the testes only a faint ghostly outline of the seminiferous tubules was recognizable, and the remaining tissue consisted of an untidy tangle of very pale-staining fibers, a few fusiform fibro-

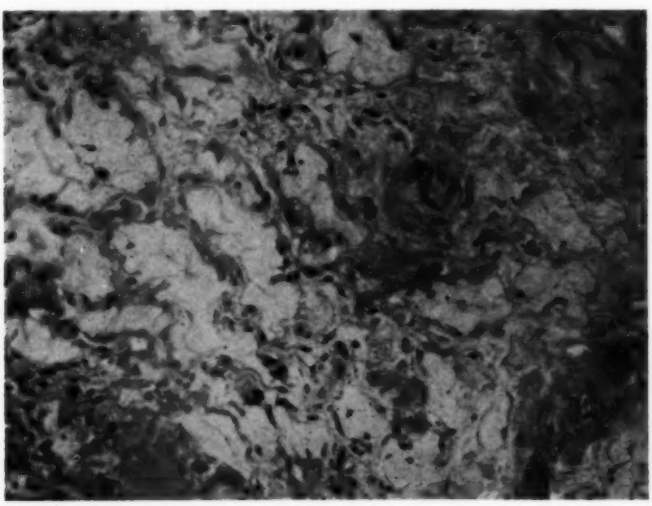


Fig. 2. — High-power view of Figure 1. No interstitial cells can be seen in the tangled connective tissue lying between the flattened faintly hyalinized seminiferous tubules. Hematoxylin and eosin; × 240.

blasts, and fairly numerous mast cells. Interstitial cells were not recognized in the hematoxylin and eosin sections, but in sections stained with Sudan III a few interstitial cells could be distinguished as small clumps of branched cells whose cytoplasm stained light orange.

The prostate had been infiltrated by tumor. The few remaining tubules were very atrophic and lined by low cuboidal epithelium. There was an early squamous metaplasia of the ducts, and most of the bulk of the gland was composed of closely set muscle fibers.

The pituitary gland was normal in size. It was stained by a modified periodic acid-Schiff (PAS) technique, and a differential count was done on a horizontal section. The histological detail and percentage of various cell types was within normal limits.

The thyroid was small, weighing 13 gm., and a few minute cysts were present on the right lower pole. The microscopic pattern was slightly nodular, the acini in these nodules being large and containing dark colloid, which was occasionally broken into small even masses. Between these nodules, the thyroid tissue was composed of tiny acini, either empty or containing clumped masses of secretion. There was no lymphoid infiltration of the stroma.

The right adrenal was completely replaced by tumor. The left adrenal was very small, and its cortex was uniform, composed of large finely vacuolated lipoid storage cells, which at places extended up to the capsule and down to the central vein. The zona reticularis appeared thin and slightly atrophic. The zona glomerulosa was abnormally wide in some places and elsewhere was absent. The adrenal medulla appeared normal. The predominant findings were lipoid storage and limited hypertrophy of the zona glomerulosa.

There was no evidence of syphilis in any of the tissues.

Comment

The number of reported cases of "true" Klinefelter's syndrome is increasing. In the past two years, 135 cases have been collected from the available literature.[4,7-9] The patient usually presents as a problem of gynecomastia or infertility. A report from a male-infertility clinic in Glasgow [8] revealed that 10 out of 91 cases with oligospermia or azoospermia in a two-year period were "true" Klinefelter's syndrome, and four of them showed some degree of spermatogenesis on biopsy. This increased to seven the number of reported cases of

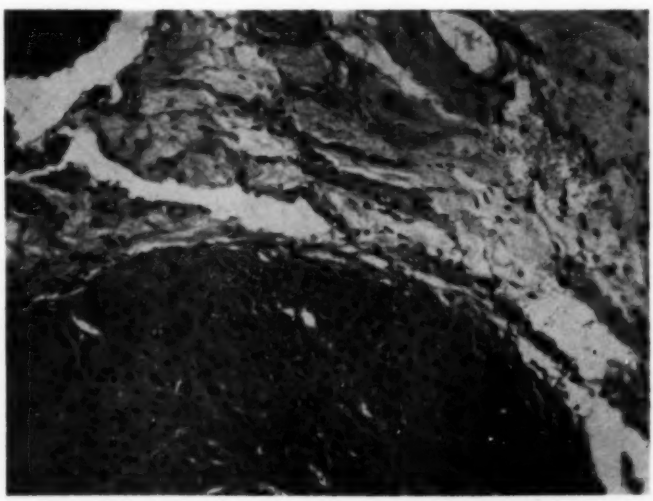


Fig. 3.—High-power view of testicular biopsy specimen from a 37-year-old man with chromatin-positive nuclei ("true" Klinefelter's syndrome). Note hyperplasia of interstitial cells (lower) and small atrophic hyalinized seminiferous tubules. Hematoxylin and eosin; × 240.

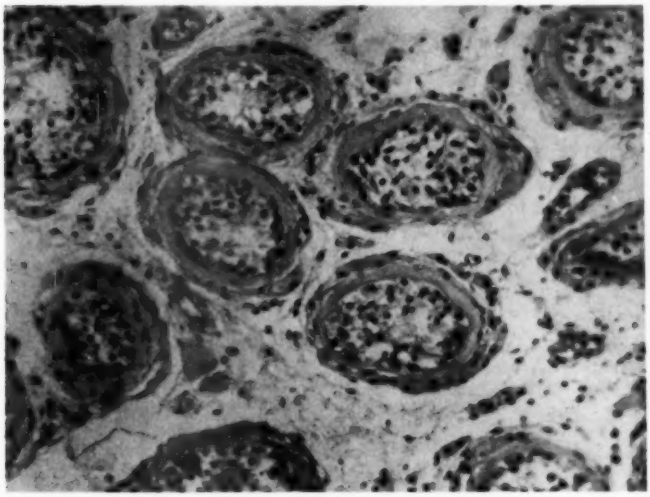


Fig. 4. — High-power view of testicular biopsy specimen from a 27-year-old man with chromatin-negative nuclei ("false" Klinefelter's syndrome). Note the normal size of the seminiferous tubules, thick basement membranes, and absence of spermatogenesis. The interstitial cells are normal but scattered.

"true" Klinefelter's cases that have shown immature or mature spermatogenesis.[5,12]

The prominence of interstitial cells has been held to be a characteristic feature of the testicular histology in Klinefelter's syndrome. Our case showed virtually no interstitial cells. Several authors[13,14] have reported that spermatogenesis may be decreased in portal cirrhosis. In these cases the testes are usually small and show focal hyalinization of seminiferous tubules and reduction in the number of interstitial cells. The testicular atrophy, as well as the associated gynecomastia, probably results from a decreased destruction of estrogens by the damaged liver. The almost complete absence of interstitial cells in our case may be explained by the co-existence of portal cirrhosis. However, the prepuberal size and complete hyalinization of the seminiferous tubules are characteristic features of "true" Klinefelter's syndrome and have not been reported, to our knowledge, in portal cirrhosis.

Clinically, it was thought that this patient's obesity and hypogonadism might be related to hypothalamic damage suffered at the time of his head injury. However, at necropsy there was no evidence of any hypothalamic or intracranial damage. His eunuchoid appearance suggests that his hypogonadism extended back at least as far as puberty.

In spite of his positive blood Wassermann test from 1941 to the time of his death, there is no clinical or pathological indication that syphilis played any part in his condition. "True" Klinefelter's cases usually show normal heterosexual interest and activity, and a recent psychiatric report[15] noted no increase in homosexuality. It is likely that this man was active sexually and contracted his syphilis in this manner.

We know of no description of the changes that occur with advanced years in the testes of patients with "true" Klinefelter's syndrome. However, Sniffen[16] described several hundred normal testes of patients over 50 years of age and noted no case with absence of interstitial cells. It seems unlikely that the patient's age alone could account for the virtual absence of interstitial cells in our case.

Summary

The clinical and pathological features of "true" Klinefelter's syndrome are reviewed and contrasted with "false" Klinefelter's syndrome. In all other reported cases of "true" Klinefelter's syndrome, testicular

histology has shown perivascular clumps of interstitial cells and small uniformly hyalinized seminiferous tubules. An autopsied case of "true" Klinefelter's syndrome with almost complete absence of interstitial cells in the testes is described. The unusual testicular histology in this case is ascribed to the coexistence of cirrhosis of the liver.

Department of Pathology, University of Toronto, 100 College St. (5).

REFERENCES

1. Klinefelter, H. F., Jr.; Reifenstein, E. C., Jr., and Albright, F.: Syndrome Characterized by Gynecomastia, Aspermatogenesis Without A-Leydism, and Increased Excretion of Follicle Stimulating Hormone, J. Clin. Endocrinol. 2:615-627 (Nov.) 1942.

2. Nelson, W. O., and Heller, C. G.: Hyalinization of Seminiferous Tubules Associated with Normal or Failing Leydig-Cell Function: Microscopic Picture in Testis and Associated Changes in Breast, J. Clin. Endocrinol. 5:13-26 (Jan.) 1945.

3. Barr, M. L., and Bertram, E. G.: A Morphological Distinction Between Neurones of the Male and Female, and the Behaviour of the Nucleolar Satallite During Accelerated Nucleoprotein Synthesis, Nature, London 163:676-677 (April 30) 1949.

4. Nelson, W. O.: Sex Differences in Human Nuclei with Particular Reference to the "Klinefelter Syndrome," Gonadal Agenesis and Other Types of Hermaphroditism, Acta endocrinol. 23: 227-245 (Nov.) 1956.

5. Witschi, E.; Nelson, W. O., and Segal, S. J.: Genetic, Developmental and Hormonal Aspects of Gonadal Dysgenesis and Sex Inversion in Man, J. Clin. Endocrinol. 17:737-753 (June) 1957.

6. Danon, M., and Sachs, L.: Sex Chromosomes and Human Sexual Development, Lancet 2:20-25 (July 6) 1957.

7. Riis, P.; Johnsen, S. G., and Mosbech, J.: Nuclear Sex in Different Types of Severe Male Hypogonadism, Lancet 2:162-164 (July 27) 1957.

8. Ferguson-Smith, M. A.; Lennox, B.; Mack, W. S.; and Stewart, J. S. S.: Klinefelter's Syndrome: Frequency and Testicular Morphology in Relation to Nuclear Sex, Lancet 2:167-169 (July 27) 1957.

9. Barr, M. L.: The Sex Chromatin and Its Bearing on Errors of Sex Development, Canad. M. A. J. 74:419-422 (March 15) 1956.

10. Segal, S. J., and Nelson, W. O.: Developmental Aspects of Human Hermaphrodism: The Significance of Sex Chromatin Patterns, J. Clin. Endocrinol. 17:676-692 (May) 1957.

11. Barr, M. L.: Dysgenesis of the Seminiferous Tubules, Brit. J. Urol. 29:251-257 (Sept.) 1957.

12. Bunge, R. G., and Bradbury, J. T.: Newer Concepts of Klinefelter's Syndrome, J. Urol. 76: 758-765 (Dec.) 1956.

13. Barr, R. W., and Sommers, S. C.: Endocrine Abnormalities Accompanying Hepatic Cirrhosis and Hepatoma, J. Clin. Endocrinol. 17: 1017-1029 (Sept.) 1957.

14. Lloyd, C. W., and Williams, R. H.: Endocrine Changes Associated with Laennec's Cirrhosis of Liver, Am. J. Med. 4:315-330 (March) 1948.

15. Pasqualini, R. Q.; Vidal, G., and Bur, G. E.: Psychopathology of Klinefelter's Syndrome: Review of 31 Cases, Lancet 2:164-167 (July 27) 1957.

16. Sniffen, R. C.: The Testis: The Normal Testis, Arch. Path. 50:259-284 (Sept.) 1950.

Experimental Virus Hepatitis in Choline-Deficient Mice with Fatty Livers

BORIS RUEBNER, M.D.; JAMES L. BRAMHALL, M.B., Ch.B., and GERALD R. BERRY, B.A., B.Sc.,
Halifax, N. S., Canada

A fatty liver has long been thought to be predisposed to further damage by other hepatotoxic agents (Opie and Alford, 1915). It is also generally recognized that experimental animals with fatty livers tend eventually to develop hepatic cirrhosis (Himsworth, 1950). A similar sequence may perhaps occur in man (Popper, 1955). A fatty liver is, therefore, often considered to be functionally impaired.

This investigation was undertaken in order to determine whether mice with fatty livers due to a choline-deficient diet had a greater susceptibility to experimental virus hepatitis than mice given the same diet supplemented with choline. Several experiments were performed with similar results. One experiment in which the number of mice was sufficient for statistical analysis is described below.

Material and Methods

Mice.—Eighty mice (forty males and forty females) aged 21 days of the Swiss Webster strain, supplied by Budd Mountain Farm, Chester, N. J., were used. Examination of peripheral blood films showed these mice to be free from Eperythrozoon coccoides.

Virus.—Mouse hepatitis virus strain MHV3 (Dick, Niven, and Gledhill, 1956) was chosen. It is uniformly fatal to weanling mice even in the absence of E. coccoides, which is required by some viruses of this group for the full expression of pathogenicity. The mortality due to this virus decreases with age and is approximately 30% at 32 days in the Budd Mountain Farm Swiss Webster mouse when fed a standard laboratory diet.

Submitted for publication Feb. 19, 1958.

From the Department of Pathology, Dalhousie University. Mr. Berry was in receipt of a Lederle of Canada Student Research Fellowship during this investigation.

Diets.—Two diets were used. Diet A (choline deficient) was similar to that of Glynn and Himsworth (1944) except for the addition of alpha tocopherol in order to prevent massive hepatic necrosis. This diet consisted of the following ingredients:

	%
Lard *	50
Cod liver oil	1
Salt Mixture No. 2, U. S. P.	3
Vitamin mixture (Williams & Aronsohn, 1956) †	1
Corn starch ‡	29
Casein §	16
Alpha tocopherol	0.01

Diet B was identical with Diet A except for the addition of 0.5% choline.

Experimental Methods.—The mice were divided into two groups of 40. One group was fed an unrestricted quantity of Diet A, and the other was similarly offered Diet B.

Five animals were kept in each cage. Unlimited fresh water was supplied daily. The groups were weighed weekly.

After 11 days 28 mice given Diet A and 29 mice given Diet B were given intraperitoneal injections of 0.1 ml. of a 10% suspension of mouse liver obtained from mice infected with MHV3 virus. The remaining mice acted as controls, and on the 11th day liver biopsy was performed on two mice from each group.

After the injection was given the cages were inspected twice daily, and any dead mice found were removed for postmortem examination.

On the 14th day after injection one mouse from each group which had survived the hepatitis was killed for histological examination.

Specimens of liver for histological study were taken from every animal. Some mice were sub-

* Supplied by Canada Packers Ltd., Toronto.

† Thiamine, 0.05%; riboflavin, 0.025%; calcium pantothenate, 0.1%; pyridoxine, 0.02%; nicotinic acid, 0.02%; powdered sugar, 99.705%.

‡ Supplied by Canadian Starch Company Ltd., Toronto.

§ Supplied by Mead Johnson & Company, Ltd., Toronto.

jected to a full histological examination. The blocks were fixed with corrosive sublimate and formalin. Paraffin sections were stained with hematoxylin and eosin and many by Van Gieson's method and by Lendrum's (1957) method for reticulin. Frozen sections for fat were stained with Sudan III.

Peritoneal swabs from 11 mice were cultured for organisms.

Results

The animals of both groups grew almost as well as animals on a standard laboratory diet. Mice on Diet B gained rather more than those on Diet A, but the difference in average weight was small. Two deaths occurred in Group A during the early period of the choline-deficient diet, and a single similar death took place in Group B.

Liver biopsy specimens taken just before injection of the virus revealed that the mice given Diet A had very fatty livers. Fatty cysts were well marked, particularly in the centrilobular areas (Fig. 1). Mice in Group B, however, showed no stainable fat (Fig. 2). In a previous experiment two mice in each group had been killed and the fat contents of the liver analyzed. The results were as follows: fat in fresh weight of liver from Group A, 21%; fat in fresh weight of liver from Group B, 3.3%.

The animals appeared normal until the third day after the injection. At that time some of the animals became listless and weak. They huddled together in the corners of their cages. A few appeared to have yellow sclerae. On the fourth day the first deaths occurred, and the Table shows the number of deaths occurring on

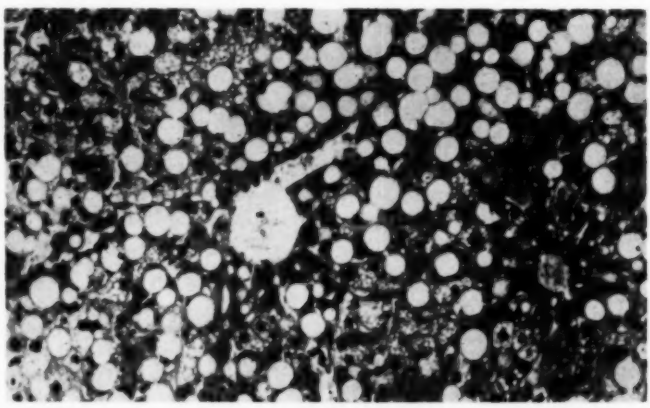


Fig. 1.—Liver biopsy specimen from a mouse which had been given the choline-deficient diet (Diet A) for 11 days. There is marked fatty change, predominantly centrilobular. Hematoxylin and eosin; reduced 28% from mag. × 300.

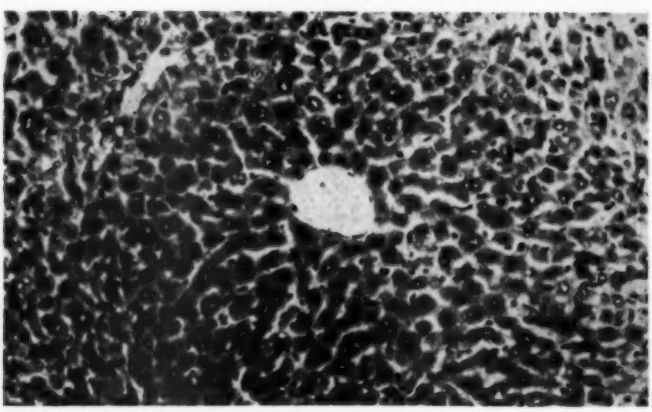


Fig. 2.—Liver biopsy specimen from a mouse which had been given the choline-supplemented diet (Diet B) for 11 days. There is no fatty change. Hematoxylin and eosin; reduced 28% from mag. × 300.

The Number of Mice Dying Each Day After the Injection of Hepatitis Virus

	Day												Total Deaths
	1	2	3	4	5	6	7	8	9	10	11	12	
Mice given Diet A	0	0	0	5	6	8	0	2	0	0	0	0	21
Mice given Diet B	0	0	0	3	8	5	3	2	0	0	0	0	21

each day after injection. There was clearly no difference in mortality between the two groups. In each group the mortality of both sexes was similar. No deaths occurred after the eighth day, and within a few days the mice became clinically normal.

At postmortem examination the gross appearance of the liver in the two groups was very different. Mice in Group A had greatly enlarged pale livers, while mice in Group B had very much smaller rather darker livers. In both groups yellow areas of necrosis and a few pinhead hemorrhagic areas could often be seen on the surface of the liver, and some of the mice showed a scanty peritoneal exudate consisting of yellow fluid.

Histologically all mice showed a massive eosinophilic hepatic necrosis which involved the greater part of the surface of the liver and spared only a few hepatic cells. A scanty inflammatory exudate was present. The surviving cells were irregularly distributed throughout the liver, some being periportal and some, centrilobular. They had a basophilic cytoplasm. Multinucleated hepatic cells and mitotic figures were extremely rare. Mice which had been given Diet A showed marked fatty change in addition to the areas of necrosis (Fig. 3). No definite anatomical relationship between the fatty change and the necrosis could be discerned in this group. The mice given the choline-supplemented Diet B did not show any stainable fat (Fig. 4). In both groups small necrotic areas were present also in the spleen and omental fat. No constant histological changes were seen in other organs. The mice killed on the 14th day after injection showed almost complete regeneration of the hepatic parenchymal cells. In Group A the regenerated cells contained much less fat than the surviving cells.

No organisms of the Salmonella group were isolated from any of the 11 peritoneal swabs taken at autopsy. Three specimens were sterile. Normal intestinal organisms only were isolated from the other swabs.

Comment

The pathological changes observed in this investigation were very similar to those previously described both in mouse hepatitis (Dick, Niven, and Gledhill, 1956) and

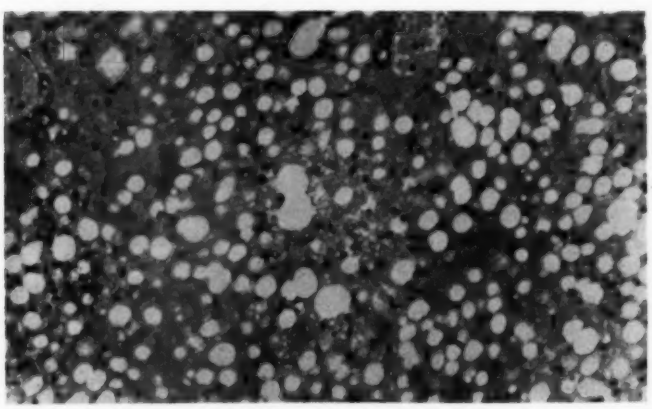


Fig. 3.—Liver specimen obtained at postmortem examination from a mouse given Diet A, which died from hepatitis five days after injection of the virus. There is hepatic necrosis and fatty change. Hematoxylin and eosin; reduced 28% from mag. × 300.

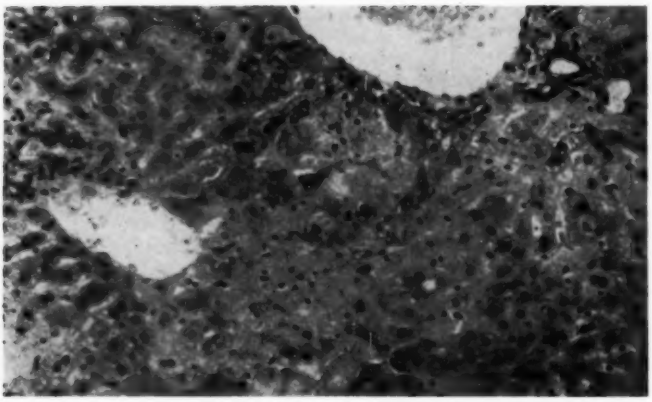


Fig. 4.—Section of liver obtained at postmortem examination from a mouse given Diet B, which died from hepatitis five days after injection of the virus. There is hepatic necrosis but no fatty change. Hematoxylin and eosin; reduced 28% from mag. ×300.

in choline deficiency (Meader and Williams, 1957). The principal finding of this study was that the fatty liver of choline deficiency was no more susceptible to necrosis produced by MHV3 virus than the histologically normal liver of the choline-supplemented animals.

It is generally accepted that deficient diets may predispose the liver to injury by other noxious agents (Waterlow and Bras, 1957). Most of the previous experiments combining dietary deficiency and other hepatotoxic agents (e. g., Miller and Whipple, 1940) have employed protein deficiency rather than choline-deficient diets. Goldschmidt, Vars, and Ravdin (1939) found that a fatty liver was more susceptible to chloroform poisoning than a normal liver, but their animals were also suffering from protein deficiency. Heppel, Porterfield, and Sharpless (1947) investigated the effect of choline deficiency on poisoning by ethylene dichloride. Addition of choline to their experimental diet prevented the development of a fatty liver but did not affect the mortality. This result is similar to ours. Animals given their experimental diets did, however, have an increased mortality compared with that of mice given a standard laboratory diet. When cystine was added to their diets the mortality became comparable to that of animals given an ordinary diet. In mouse hepatitis also, although choline deficiency has no effect on mortality, Ruebner and Bramhall (1958) found that mice given a standard laboratory diet were much more resistant than mice on Diets A and B. They concluded that this result was due to the lack of some dietary factor, possibly a protein or amino acid, such as cystine.

The conclusion from our experiments seems to be that the mere presence of fatty change and even of fatty cysts in the liver may not necessarily be of great functional importance. This is supported by the findings of Sutherland (1956) that in the rat fatty livers regenerate as well as normal livers. Much work has recently been done on kwashiorkor, a human deficiency syndrome. A fatty liver is a prominent feature of this condition. The degree of fatty change, however, seems to bear little relation to the clinical severity of the disease, and there is little disturbance of liver-function tests (Waterlow and Bras, 1957). Moreover, there appears to be no unequivocal causal relationship between fatty change and hepatic fibrosis in this condition (Gillman and Gilbert, 1954). These findings, like our own, suggest that fatty metamorphosis may not be as important a predisposing factor to further liver damage as was previously believed.

Few controlled studies have been published about the effect of diet in human hepatitis. Dible, McMichael, and Sherlock (1952) found that a patient with severe

hypoproteinemia may recover fully from this disease. There are, however, observations suggesting that malnutrition may greatly increase the mortality in epidemics of hepatitis (Himsworth, 1950). In experimental mouse hepatitis also a good diet has a protective effect (Ruebner and Bramhall, 1958). Our present results, however, suggest that fatty change in the human liver may not be harmful in virus hepatitis.

Summary

The effects of a choline-deficient diet on mouse virus hepatitis due to MHV3 was investigated.

Two groups of mice were used. One group of 28 mice was given the experimental diet. Twenty-nine other mice were fed a similar diet to which 0.5% choline had been added. After 11 days on their respective diets both groups were given intraperitoneal inoculations of 0.1 ml. of a liver suspension containing the virus. The number of deaths in both groups was identical (21).

It is concluded that the fatty liver of choline deficiency is no more susceptible to virus hepatitis than the histologically normal liver of the choline-supplemented animals.

The mere presence of fatty change in the liver, therefore, may not necessarily be of great functional importance.

Dr. A. W. Gledhill supplied the MHV3 and gave advice in this study. Dr. W. A. Taylor provided encouragement and support and Dr. F. Moya did the chemical analysis.

Department of Pathology, Pathological Institute, Dalhousie University, 62 University Ave.

BIBLIOGRAPHY

Dible, J. H.; McMichael, J., and Sherlock, S.: Serum Hepatitis with Recovery in Severe Protein Deficiency, Lancet 1:999, 1952.

Dick, G. W. A.; Niven, J. S. F., and Gledhill, A. W.: A Virus Related to That Causing Hepatitis in Mice (MHV), Brit. J. Exper. Path. 37:90, 1956.

Gillman, J., and Gilbert, C.: Aspects of Nutritional Liver Disease—Human and Experimental, Ann. New York Acad. Sc. 57:737, 1954.

Glynn, L. E., and Himsworth, H. P.: Massive Hepatic Necrosis and Diffuse Hepatic Fibrosis (Acute Yellow Atrophy and Portal Cirrhosis): Their Production by Means of Diet, Clin. Sc. 5:93, 1944.

Goldschmidt, S.; Vars, H. M., and Ravdin, I. S.: The Influence of Foodstuffs upon the Susceptibility of the Liver to Injury by Chloroform and the Probable Mechanisms of Their Action, J. Clin. Invest. 18:277, 1939.

Heppel, L. A.; Porterfield, V. T., and Sharpless, N. E.: Toxicology of 1:2 Dichlorethane (Ethylene Dichloride): IV. Its Detoxification by l-Cystine, dl-Methionine and Certain Other Sulphur Containing Compounds, J. Pharmacol. & Exper. Therap. 91:885, 1947.

Himsworth, H. P.: Lectures on the Liver and Its Diseases, Ed. 2, London, Oxford University Press, 1950.

Lendrum, A. C.: Personal communication to the authors, 1957.

Meader, R. D., and Williams, W. L.: Choline Deficiency in the Mouse, Am. J. Anat. 100: 167, 1957.

Miller, L. L., and Whipple, G. H.: Chloroform Liver Injury Increases as Protein Stores Decrease: Studies in Nitrogen Metabolism in These Dogs, Am. J. M. Sc. 199:204, 1940.

Opie, E. L., and Alford, L. B.: The Influence of Diet upon Necrosis Caused by Hepatic and Renal Poisons: Part I. Diet and the Hepatic Lesions of Chloroform, Phosphorus or Alcohol, J. Exper. Med. 21:1, 1915.

Popper, H.; Szanto, P. B., and Elias, H.: Transition of Fatty Liver into Cirrhosis, Gastroenterology 28:183, 1955.

Ruebner, B., and Bramhall, J.: Unpublished observations, 1958.

Sutherland, A. M.: Regeneration in the Fatty Liver of the Rat After Partial Hepatectomy, J. Path. & Bact. 71:403, 1956.

Waterlow, J. C., and Bras, G.: Nutritional Liver Damage in Man, Brit. M. Bull. 13:107, 1957.

Williams, W. L., and Aronsohn, R. B.: Cardiac and Hepatic Lesions in Mice Fed Yeast-Protein Diets: I. Diets Containing British Bakers Yeast, Yale J. Biol. & Med. 28:515, 1956.

Fatal Toxic Hepatitis Following Chlorpromazine Therapy

Report of a Case with Autopsy Findings

A. E. RODIN, M.D., and D. M. ROBERTSON, M.D., C.M., Kingston, Ont., Canada

Jaundice as evidence of liver injury is the commonest manifestation of untoward reaction in patients treated with chlorpromazine,* [1] aside from those directly related to the pharmacological action of this drug.[2] The incidence of jaundice has been reported as from 0.2% to 8% [4] of cases and is invariably obstructive in nature.[5] Although recovery is the rule, there have been sufficient liver biopsies reported to ascertain a definite pattern of abnormal histological changes in the liver—the so-called intralobular cholestasis.[6]

However, autopsy findings in fatalities associated with chlorpromazine jaundice are not well known, since up to the present time there have been only two reported deaths with autopsies.[7,8] As there is a rarity of autopsy findings, and as both liver biopsy and autopsy slides are available in the present case, a report of the findings may provide information that will increase our knowledge of the pathogenesis of chlorpromazine jaundice.

Report of Case

History.—A 43-year-old woman was admitted to the Ontario Mental Hospital, in Kingston, on May 25, 1956, the diagnosis being involutional depression with agitation. There was no significant past history and no record of allergy or drug sensitivity. Physical examination was negative except for a blood pressure of 160/100. Both electrocardiographic and electroencephalographic studies were negative. Because of marked excitement, chlorpromazine therapy, 50 mg. q. i. d., intramuscularly, was begun on May 28, but changed to 50 mg. by mouth on June 1. She became less agitated on this therapy, and the drug was discontinued on June 6, after nine days of therapy, the total dose being 1800 mg.

On June 19, twenty-two days after the onset of chlorpromazine therapy, and thirteen days after it was discontinued, the patient began to complain of anorexia, flatulence, and fatigue. Jaundice was noted, together with clay-colored stools and copper-colored urine, but pain was absent. Laboratory examinations on June 25 revealed a total serum protein of 5.8 mg. %, with an albumin-globulin ratio of 0.8, an N. P. N. value of 16 mg. %, a negative cephalin-cholesterol flocculation test, and a serum alkaline phosphatase value of 78 K. A. units. Because of the clinical picture of increasing obstructive jaundice, a laparotomy was performed on July 12, twenty-four days after the onset of jaundice. However, at operation, the liver, extrahepatic biliary system, and pancreas were thought to be normal. A liver biopsy was performed.

Postoperatively, the jaundice became progressively severer and the patient's condition deteriorated. She became very agitated and resistant to care. On July 30, forty days after the onset of jaundice, shock and cyanosis became pronounced and the patient died. During her hospital stay, the only additional drugs received were phenobarbital (Luminal), sodium amobarbital (Amytal) and paraldehyde, and morphine, shortly before death.

Pathological Findings

Liver Biopsy

The surgical specimen consisted of an elliptical piece of liver tissue measuring 2×1.5×1.5 cm., with a smooth glistening capsular surface. The cut surface was a homogeneous pale yellow, except for the areas immediately surrounding the central

Submitted for publication Feb. 19, 1958.

From the Departments of Pathology and Psychiatry, Queen's University and the Kingston General Hospital. Present address of Dr. Rodin: Department of Pathology, Deer Lodge Hospital, Winnipeg 12, Man.

* Trade names: Largactil, Thorazine.

veins, which presented as small dark foci. Microscopic sections of the liver showed good preservation of the architectural pattern. However, the central vein areas were well defined, owing to early degenerative changes in adjacent hepatic cells and increased amounts of bile pigment in these and the Kupffer cells (Fig. 1). Parenchymatous changes consisted of cytoplasmic swelling, mild vacuolation, and early necrosis. Many of the bile canaliculi were distended with bile plugs, this change being restricted to centrilobular areas (Fig. 2). The portal triads showed a moderate chronic inflammatory-cell infiltrate, consisting of lymphocytes with a scattering of plasma cells (Fig. 3). The middle and peripheral zones of the lobules were not unusual.

Autopsy Findings

At autopsy, 11 hours after death, external examination showed a thin and extremely jaundiced woman who appeared to be considerably older than her stated age. A slight amount of pitting edema of the lower extremities was present. The abdomen contained 1000 cc. of slightly reddish-stained fluid, and the right and left pleural spaces contained 300 and 400 cc., respectively, of straw-colored fluid. The lower lobes of both lungs were

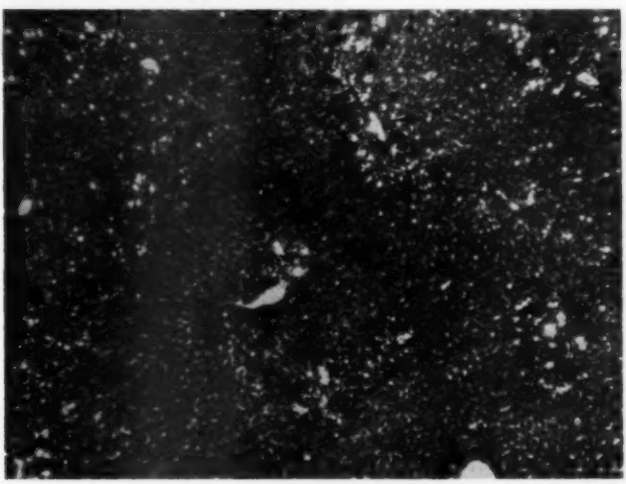


Fig. 1.—Liver biopsy specimen. Centrilobular degeneration, with preservation of lobular architecture. Pigment and bile thrombi are concentrated in the centrilobular regions. Hematoxylin—phloxine—saffron; ×90.

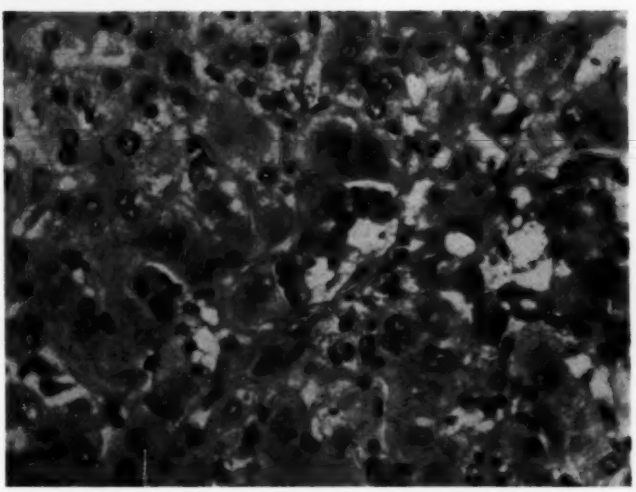


Fig. 2.—Liver biopsy specimen. Centrilobular region, showing degenerative changes and minimal necrosis of parenchymal cells, bile thrombi, and pigment in liver and Kupffer cells. Hematoxylin — phloxine—saffron; × 400.

Fig. 3.—Liver biopsy specimen. Portal area, showing marked infiltration by lymphocytes and plasma cells. The bile ducts are not dilated and do not contain inspissated bile. Hematoxylin—phloxine—saffron; ×220.

dark and displayed diminished crepitus, and a small amount of fluid was expressable from the cut surface. The kidneys were enlarged (175 gm. each), with smooth pale capsules and pale cortices, measuring 6 mm. However, the renal medullae were streaked with green pigment.

The liver at autopsy was firm and enlarged, weighing 1950 gm. The capsular surface was smooth with greenish mottling. Cut surface revealed a marked greenish-yellow discoloration with darker greenish-brown centrilobular areas. The biliary tract was patent throughout, with a lumen of normal size, and no abnormalities of gallbladder or pancreas were found. No other pertinent gross findings were present.

Microscopic examination revealed, in addition to hepatic changes, acute bilateral bronchopneumonia and cholemic nephrosis.

Microscopic examination of the liver showed much more extensive damage than the surgical biopsy specimen, with marked necrosis of the hepatic cells in the central one-half of two-thirds of all lobules (Fig. 4). There were varying degrees of hepatic-cell necrosis, ranging from complete loss of structure to nuclear pyknosis and indistinct cytoplasmic outlines. How-

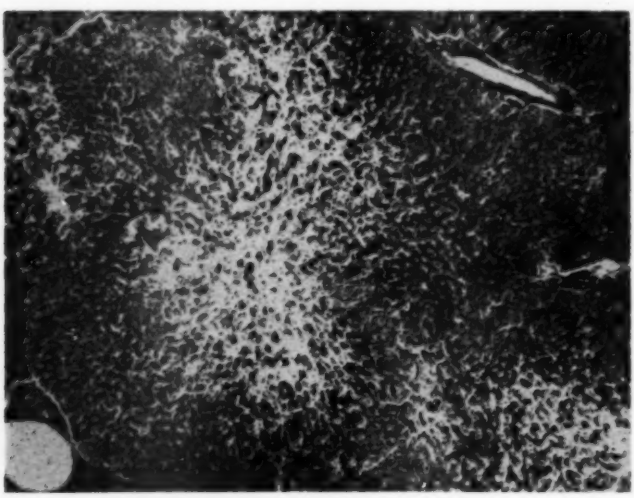


Fig. 4.—Autopsy liver specimen. Centrilobular necrosis involving one-half to two-thirds of lobule. Hematoxylin—phloxine— saffron; ×90.

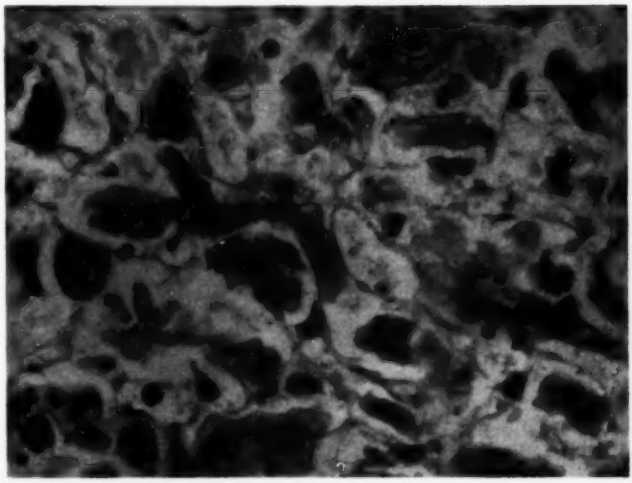


Fig. 5.—Autopsy liver specimen. Centrilobular region. There is marked necrosis of parenchymal cells, with preservation of the supporting reticulum. Bile thrombi, and pigment within seen. Hematoxylin—phloxine—saffron; × 400.

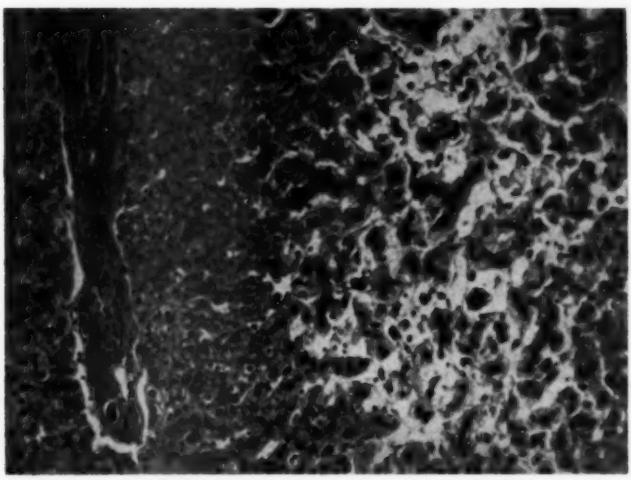


Fig. 6.—Autopsy liver specimen. Periphery of lobule. Parenchymal cells are somewhat swollen and show granularity. The portal area is infiltrated by moderate numbers of lymphocytes. Hematoxylin—phloxine—saffron; × 220.

ever, the reticulum network was preserved (Fig. 5). The cells in the periphery of the lobules, although intact, showed slight swelling, vacuolation, and increased granularity (Fig. 6). No evidence of cellular regeneration was found. Intracanalicular bile thrombi, similar to those seen in the surgical slide, were still present and still limited to the central areas of the lobules. Intracellular bile pigment was present, but not conspicuous. The portal triads showed somewhat less cellular infiltration than the surgical specimen and no additional fibrosis.

Comment

The principal pathological feature of chlorpromazine jaundice, as seen in biopsy specimens of this and other reported cases, is centrilobular bile stasis. In addition, degeneration of centrally located hepatic cells and infiltration of portal tracts by lymphocytes, plasma cells, and eosinophils, conspicuous in this case, are usually, although not invariably, present. In this case, it would appear that the minimal cellular damage seen in the biopsy specimen progressed to extensive centrilobular ne-

crosis, resulting in death as a result of hepatic insufficiency. A similar pathological pattern was reported in the other two fatalities found in the literature.[7,8] Although central necrosis has been reported as an agonal phenomenon,[16] it is doubtful if the severe degree of change seen in these three cases could be explained on this basis alone.

The pathological features of chlorpromazine hepatitis as seen on biopsy specimens can readily be distinguished from those of viral hepatitis[9] or toxic hepatitis due to such poisons as phosphorus and carbon tetrachloride or drugs such as sulfonamides[10] and izoniazid.[11] An identical picture is described in cases of jaundice occurring during therapy with thiouracil,[12] arsphenamine,[13] and methyltestosterone,[14] suggesting a common pathogenesis. The identity of the changes of early extrahepatic obstruction with those of chlorpromazine centrilobular cholestasis would make the differentiation of these two conditions difficult.

Although a direct toxic action of chlorpromazine on the liver has been considered,[18] most authors regard the damage as being on the basis of hypersensitivity. The features suggestive of the latter include the lack of relationship between dosage and occurrence of jaundice, the long latent period, the occurrence of jaundice after cessation of therapy,[20,21] the small number of those receiving chlorpromazine who are affected, and the rapid response to corticotropin (ACTH).[9] Eosinophilic or plasma cellular infiltrations in the portal areas have been described in many cases, and plasma cells were conspicuous in this case.

The mechanism by which the clinical picture of obstructive jaundice is produced is not apparent from histological examination of the lesions. Centrilobular bile stasis and mild cellular damage have been reported in early cases of extrahepatic biliary obstruction,[15] and chlorpromazine is known to produce a transient rise in bile duct pressure, presumably as a result of spasm of the sphincter of Oddi.[17] However, the absence of duct dilatation and of other evidence of obstruction 40 days from the onset of jaundice makes this mechanism most unlikely. It has been suggested that intralobular bile canaliculi may be obstructed by swollen liver cells or by abnormal, more viscid bile excreted by the damaged parenchyma. It is apparent, however, that the periportal areas are free of bile thrombi, and the cells in these sites show no histological evidence of damage early in the disease. It is possible that involvement of the interlobular ducts by pericholangitis may result in obstruction.[19] This feature, however, is often minimal or absent. It is clear that injury of liver cells must be greater than is demonstrable by the methods used in this study, whatever be the mechanism of the obstructive jaundice.

Summary

A case of fatal toxic hepatitis associated with chlorpromazine therapy is reported, there being only two similar case reports found.

The similarity of chlorpromazine jaundice to early biliary obstruction and to arsphenamine, thiouracil, and methyltestosterone jaundice is noted.

Various theories as to the pathogenesis of chlorpromazine liver injury and jaundice are considered.

Department of Pathology, Deer Lodge Hospital, Winnipeg 12, Man.

REFERENCES

1. Meyer, J. H.; Kinross-Wright, V., and Finney, R. M.: Chlorpromazine as a Therapeutic Agent in Clinical Medicine, A. M. A. Arch. Int. Med. 95:202, 1955.

2. Garmany, G.; May, A. R., and Folkson, A.: The Use and Action of Chlorpromazine in Psychoneurosis, Brit. M. J. 2:439, 1954.

3. Moyer, J. H.; Kent, B.; Knight, R. W.; Morris, G.; Dizon, M.; Rogers, S., and Spurr, C.: Clinical Studies of an Anti-Emetic Agent, Chlorpromazine, Am. J. M. Sc. 228:174, 1954.

4. Azima, H., and Ogle, W.: Effect of Largactil in Mental Syndromes, Canad. M. A. J. 71:116, 1954.

5. Zatuchni, J., and Miller, G.: Jaundice During Chlorpromazine Therapy, New England J. Med. 251:1003, 1954.

6. Diseases of the Liver, edited by L. Schiff, Philadelphia, J. B. Lippincott Company, 1956, p. 360.

7. Boardman, R. H.: Fatal Case of Toxic Hepatitis Implicating Chlorpromazine, Brit. M. J. 2:579, 1954.

8. Earl, B. V.: Toxicity of Chlorpromazine, Correspondence, Brit. M. J. 1:1156, 1955.

9. Wood, D. A.: Pathologic Aspects of Acute Epidemic Hepatitis, with Especial Reference to Early Stages: Report of Series of 10 Cases, Including Case in Which There Was Spontaneous Rupture of Spleen and 6 Cases of Fulminating Disease in Patients Who Had Been Wounded Several Months Previously, Arch. Path. 41:345, 1946.

10. More, R. H.; McMillan, G. C., and Duff, G. L.: The Pathology of Sulfonamide Allergy in Man, Am. J. Path. 22:703, 1946.

11. Randolph, H., and Joseph, S.: Toxic Hepatitis with Jaundice Occurring in a Patient Treated with Isoniazid, J. A. M. A. 152:38, 1953.

12. Gargill, S. L., and Lesses, M. F.: Toxic Reactions to Thiouracil: Report of Cases with One Fatality, J. A. M. A. 127:890, 1945.

13. Hanger, F. M., Jr., and Gutman, A. B.: Postarsphenamine Jaundice Apparently Due to Obstruction of Intrahepatic Biliary-Tract, J. A. M. A. 115:263, 1940.

14. Almaden, P. J., and Ross, S. W.: Jaundice Due to Methyl Testosterone Therapy, Ann. Int. Med. 40:146, 1954.

15. Popper, H., and Franklin, M.: Diagnosis of Hepatitis by Histologic and Functional Laboratory Methods, J. A. M. A. 131:230, 1948.

16. Menguy, R. B.; Grindlay, J. H., and Cain, J. C.: The Effects of Chlorpromazine Hydrochloride on the Dynamics of the Biliary Tree, Gastroenterology 30:752, 1956.

17. Wallach, H. F., and Popper, H.: Central Necrosis of the Liver, Arch. Path. 49:33, 1950.

18. Werner, S. C.; Hanger, F. M., and Kritzler, R. A.: Jaundice During Chlorpromazine Therapy, Am. J. Med. 18:325, 1955.

19. Lemire, R. E., Jr., and Mitchell, R. A.: Regurgitation Type of Jaundice During Prolonged Therapy with Chlorpromazine, A. M. A. Arch. Int. Med. 95:840, 1955.

20. Van Ommen, R. A., and Brown, C. H.: Obstructive-Type Jaundice Due to Chlorpromazine (Thorazine): Report of 3 Cases, J. A. M. A. 157:321, 1955.

21. Kelsey, J. R.; Moyer, J. H.; Brown, W. G., and Bennett, H. D.: Chlorpromazine Jaundice, Gastroenterology 29:865, 1955.

Evaluation of Pulmonary Megakaryocytes

J. GEORGE SHARNOFF, M.D., and EUN SOOK KIM, M.D., Mount Vernon, N. Y.

The studies reported here were prompted by the observation of several investigators,[1-7] including one of us,[8] that cells presumed, on morphological evidence, to be megakaryocytes were noted with some frequency in the pulmonary vessels, chiefly the capillary beds, in some instances of thrombotic thrombocytopenic purpura. Since one of us (J. G. S.) is also of the opinion, based on the above-mentioned material, that some relationship exists between the supposed megakaryocytes and the formation of platelet thrombi, a more thorough investigation of this phenomenon appeared warranted. This was further emphasized when a review of the literature disclosed incomplete information concerning this phenomenon, with all observations also based on morphological evidence. Thus, Aschoff[9] is credited with first observing megakaryocytes in human lungs in 1893. He considered it an embolic process associated with abnormal hematopoietic activity. Later Goroncy,[10] also interpreting this as an embolic process associated with disease, observed the cells believed to be megakaryocytes in the lungs in 14 instances of 51 autopsies of humans. More recently Brill and Halpern[11] noted the presumed megakaryocytes in the lungs of all 50 human autopsies studied of persons dying from a variety of diseases. Also, Seebach and Kernohan[12] observed the cells in the lungs in all instances of acute bacterial endocarditis. Howell and Donahue,[13] and later Jordan,[14] reported on lung-perfusion studies performed on cats and dogs in whose lungs these cells were also noted. The former believed the megakaryocytes were formed in the lungs from myeloblasts and that they were a possible additional source of platelet formation. Jordan questioned whether the cells were megakaryocytes. Both studies were judged to be inconclusive. Wintrobe[15] states that megakaryocytes "are normally found in the lungs" of all adult mammalians, where they are considered to be "effete" cells. From the above, it became clear that first a closer identification of these cells as megakaryocytes was desirable. Secondly, it was deemed essential to determine how frequently these cells could be observed in all lungs and, finally, that an attempt be made to determine the fate of these cells.

Submitted for publication Nov. 7, 1957.

From the Department of Pathology, Mount Vernon Hospital.

Methods and Materials

Since Wachstein,[16] Storti et al.,[17] and Wislocki et al.[18] succeeded in demonstrating the presence of polysaccharides in the megakaryocyte cytoplasm in smears or bone marrow sections by means of the periodic acid-Schiff stain, a similar demonstration was attempted on tissue section. The Hotchkiss-McManus method was used. Several lung, bone marrow, lymph node, spleen, and liver sections were studied. The latter three tissues were from a patient dying of thalassemia major and in another instance from a patient with long-standing blood loss from a leiomyosarcoma which had ulcerated the small bowel wall. In both instances hematoxylin-eosin-stained sections had revealed what were assumed to be many megakaryocytes in the lungs, liver, spleen, and lymph nodes, as well as in the bone marrow.

In a second study the lung sections of 355 persons were reviewed for the presence of megakaryocytes. This included 317 consecutive unselected human autopsies. Included in the latter were 53 neonatal deaths consisting of 13 stillborn and 40 newborn babies. The majority were prematures, with several five-month fetuses seen and the rest ranging up to full-term babies. All 53 with the exception of 4 with congenital anomalies were free of pathology. Excluding the neonatal deaths, the other autopsied persons ranged in age from 1 month to 98 years, with the average being 61.5 years. Additional lung tissues studied were of six

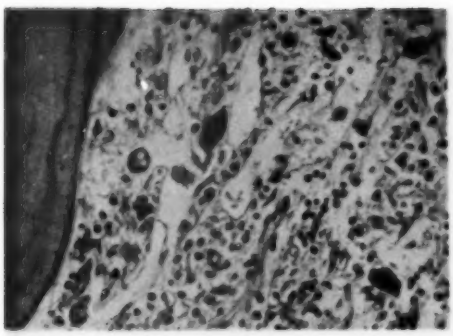


Fig. 1.—Two bone marrow megakaryocytes giving a positive reaction for polysaccharides in the cell cytoplasm. Periodic acid-Schiff stain; reduced 35% from mag. × 360.

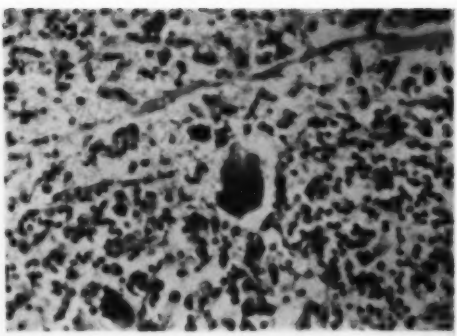


Fig. 2.—Lymph node megakaryocytes giving a positive reaction for polysaccharides in the cell cytoplasm. Periodic acid-Schiff stain; reduced 35% from mag. × 360.

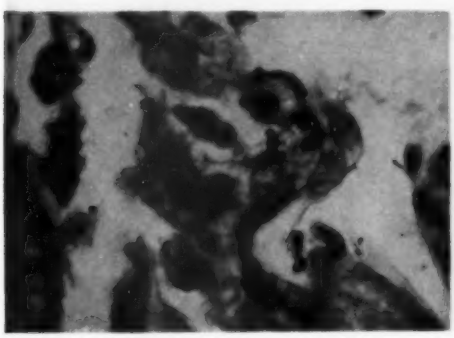


Fig. 3.—Lung megakaryocyte in a larger vessel giving a positive reaction for polysaccharides in the cell cytoplasm. Periodic acid-Schiff stain; reduced 35% from mag. × 855.

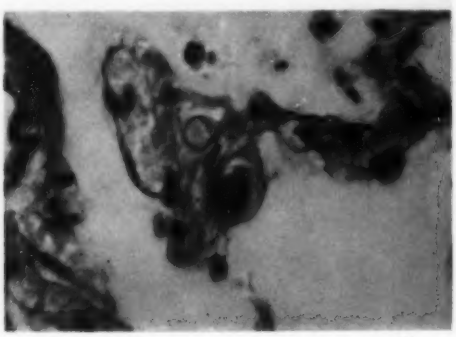


Fig. 4.—Lung megakaryocyte in a capillary vessel giving a positive reaction for polysaccharides in the cell cytoplasm. Periodic acid-Schiff stain; reduced 35% from mag. × 855.

persons who died suddenly from accidents or otherwise, with no apparent disease, and six surgically removed human lungs for neoplastic or infectious disease. The lungs of 27 mammalians of different species were also examined. These included 14 young healthy rabbits, 4 apes, 1 hog, 1 leopard, 1 mink, 1 beaver, 1 camel, 1 skunk, 1 duiker, 1 eland, and 1 squirrel. With the exception of the rabbits and squirrel, the others died of natural causes, usually pulmonary infection. All tissues examined were prepared in the usual manner of formalin fixation, embedded in paraffin, and stained with hematoxylin and eosin. Several square centimeters of lung tissue section was available for study from each animal.

In an attempt to reproduce the disappearance of the supposed megakaryocytes from the pulmonary capillary bed, as observed in three instances mentioned below, a separate study with use of rabbits as the experimental animals was undertaken. Since two of the three persons whose lungs failed to disclose the presence of megakaryocytes were given epinephrine as a stimulant shortly before death, this solution was used as the stimulating agent in this experiment. Young female Belgian hares weighing 2-4 lb. were used. Ten animals were given 10 minims of a 1:1000 epinephrine solution subcutaneously and were killed by air embolism 30 minutes later; lung tissues were removed for sectioning. Since 14 normal untreated animals as noted above revealed 2-3 megakaryocytes per square centimeter of lung tissue section, these were used as a base line control. Four animals given 1 cc. subcutaneous injections of sterile isotonic saline, also killed at 30 minutes, were used as further controls. Since epinephrine is known to elevate the platelet counts, and since it was suspected that this elevation in platelets could accompany the disappearance of the pulmonary megakaryocytes, counts were performed before and after injection on the 10 experimental and 4 saline control animals. The postinjection count was made just before the animal was killed. The platelet counts were performed by the indirect method of Fonio. This method was chosen because with direct methods the extremely small platelets could easily be overlooked.

Finally, a study was made to confirm the observations of Minot[19] that megakaryocytes may be seen with some frequency in the peripheral circulation. Twenty blood smears were examined which were prepared from the "buffy coat" of centrifuged cubital venous blood obtained from ostensibly normal humans or from persons having a local discoid chronic lupus erythematosus.

Results

With the periodic acid-Schiff stain all the cells, whether in the bone marrow, lymph nodes, spleen, liver, or lungs, presumed on morphological grounds to be megakaryocytes gave a strongly positive reaction for the presence of polysaccharides (Figs. 1, 2, and 3). Nuclear masses which appeared to be without cell cytoplasm by hematoxylin and eosin staining and presumed to be megakaryocytes squeezed into the pulmonary capillaries could be demon-

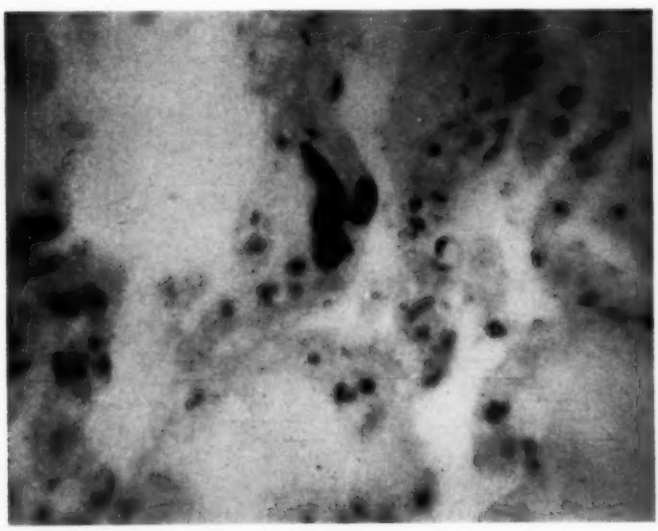


Fig. 5.—Lung megakaryocyte in a capillary vessel budding into the anastomoses of the vessel. Reduced about 15% from mag. × 800.

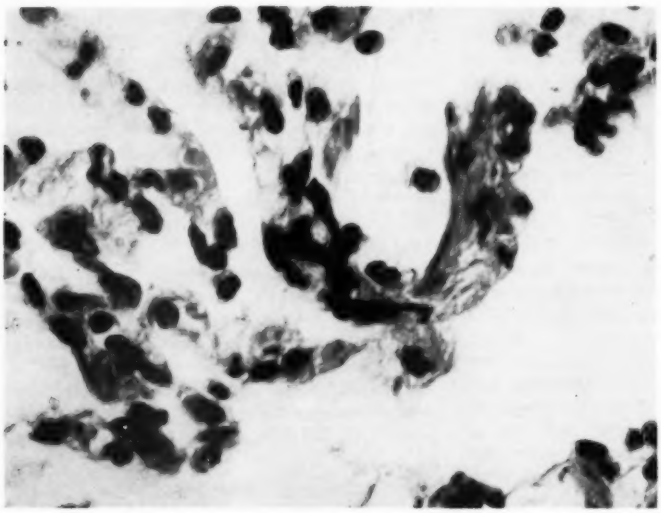


Fig. 6.—Lung megakaryocyte in the capillary of an alveolar wall apparently being broken asunder into several anastomoses. Reduced about 15% from mag. × 855.

strated as having cytoplasm with this stain technique (Fig. 4).

With the hematoxylin and eosin stain the supposed megakaryocytes were readily seen most frequently as extremely dark blue-black hematoxylin-staining large nuclear masses, appearing denuded of cytoplasm and elongated, filling and outlining the capillaries or budding into their anastomoses (Fig. 5). The nuclei were readily seen on focusing in depth through the thickness of the sections as distorted or molded by the vascular channels (Fig. 6). Less frequently, the cells were noted in the larger vessels as they appear in the bone marrow as large cells with bizarre heavily stained nuclei and relatively abundant faint eosin-staining cytoplasm (Fig. 7).

The lung sections of the 355 persons, with 3 exceptions, all revealed the above-described cells in either smaller or greater number. One exception was a 64-year-old

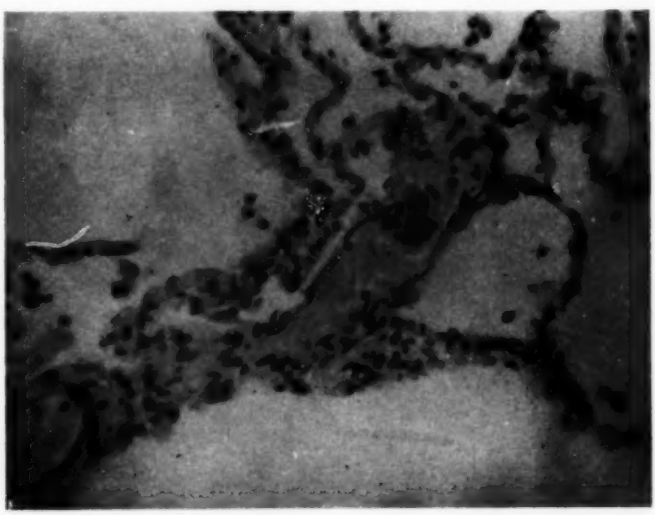


Fig. 7.—Megakaryocytes in a large pulmonary vessel. Reduced about 15% from mag. × 250. Reproduced with permission from Sharnoff, J. G.: *Am. J. Med.* 23:740-747, 1957.

man dying after being returned to his hospital bed after a laparotomy for a perforated gastric carcinoma. He had been given a 1 cc. intracardiac injection of a 1:1000 epinephrine solution in the operating room. The second exception was a 75-year-old male cardiac patient, a constant user of phenylephrine (Neo-Synephrine) for dyspnea who died suddenly of a pulmonary embolism. The third exception was a newborn baby with erythroblastosis fetalis who died shortly after an exchange transfusion.

The remaining material could be classified into three groups according to the average number of megakaryocytes seen in 1 sq. cm. of lung tissue section. In Group I were placed those with an average of 2-3 megakaryocytes; in Group II, those with an average of 5-10 megakaryocytes, and in Group III, those with 10 or more megakaryocytes per square centimeter of lung tissue section. In some instances as many as 30-40 megakaryocytes could be counted in each square centimeter of lung-tissue section. The material could be further classified according to the absence or presence of disease, with a further classification possible according to status or disease type (Table). A total of 74 subjects with no evidence of diease were classified, with 72 recorded in Group I and 2, in Group II. Included here were the 14 rabbits, 1 squirrel, 40 newborns, 13 stillborns, and the 6 adult humans who died suddenly. One newborn and one stillborn dead of exsanguination and marked hemorrhage were classified in Group II.

Some difficulty was encountered in recording findings on the autopsied humans dead of disease. This was caused by some overlapping due frequently to pneumonia, often discovered on microscopy as a terminal complication of other disease. This was considered secondary and disregarded in the classification. All the material was therefore recorded once according to the major illness or cause of death. The largest disease classification was of those persons dead of thromboembolic disease. This included instances of recent coronary thrombosis, acute myocardial infarction, pulmonary embolism with or without infarction, cerebral and mesenteric thrombosis, etc. As can be seen in the Table, of a total of 79 instances, 33 were classified in Group I; 41, in Group II, and 5, in Group III. Those recorded in Groups II and III together comprised 58.9% of the total.

The next largest diseased material contained 65 persons dead of infectious disease. Included here were instances of bacterial or viral infections, such as pneumonia, encephalitis, meningitis, peritonitis, and pyelonephritis. Excluding the 12 animals and the 4 human surgical lungs examined, 15 of the remaining 49 could be classified in Group I; 29, in Group II, and 5, in Group III. Those recorded in Groups II and III comprised 63.9% of the total.

In 57 instances designated as miscellaneous, deaths were caused by chronic heart disease, such as hypertensive cardiovascular disease and old rheumatic and syphilitic heart disease, as well as renal disease and uremia, cerebral or gastrointestinal hemorrhage, all forms of severe anemias, and cirrhosis of the liver. Forty-three were classified in Group I; ten, in Group II, and

Classification of Material

Type of Material	Total	Group I *	Group II †	Group III ‡
Normals 14 rabbits, 1 squirrel, 40 newborns, 13 stillborns, 6 adult humans (sudden or accidental deaths)	74	72 (97.3%)	2 (2.7%)	0
Thromboembolic disease	79	33 (41.1%)	41 (51.9%)	5 (7.0%)
Infectious disease (excluding 12 animals & 4 human surgicals)	49	15 (36.1%)	29 (59.2%)	5 (4.7%)
Miscellaneous	57	43 (77.2%)	10 (17.7%)	4 (5.1%)
Malignancy (excluding 2 surgicals)	35	25 (71.4%)	8 (22.8%)	2 (5.7%)
Postoperative status	36	11 (30.5%)	21 (58.3%)	4 (11.2%)
Leukemia	6	4 (66.6%)	2 (33.3%)	0

* 2-3 megakaryocytes per square centimeter.
† 5-10 megakaryocytes per square centimeter.
‡ More than 10 megakaryocytes per square centimeter.

four, in Group III. The majority of the 14 persons recorded in Groups II and III had severe anemia or died of hemorrhage and exsanguination and constituted 22.8% of the total number.

In 37 instances of malignant disease of all forms exclusive of leukemia, 27 were classified in Group I; 8, in Group II, and 2, in Group III. In one person in Group II and in both persons in Group III there were present extensive skeletal metastases.

In 36 instances of postoperative near deaths of from 1 to 31 days after major surgery, 11 were classified in Group I; 21, in Group II, and 4, in Group III. Those recorded in the latter two groups comprised 69.5% of the total.

No significant statistical information could be derived from the six cases of leukemia.

In the rabbit study, the epinephrine administration appeared to cause a striking disappearance of the megakaryocytes from the lungs. In 7 of the 10 experimental animals no pulmonary megakaryocytes could be found. In the remaining three animals a total of 4 megakaryocytes could be found in several square centimeters of lung tissue sections. The four control animals given injections of saline revealed an average of 2-3 megakaryocytes per square centimeter of lung tissue section, as did the untreated 14 normal animals. The platelet counts in the 10 epinephrine-treated animals averaged 460,000 per cubic millimeter before injection, and 690,000 per cubic millimeter after injection. The four control animals given injections of saline revealed no significant difference in the platelet counts, averaging 590,000 per cubic millimeter before injection and 610,000 per cubic millimeter after injection. No significant alteration in erythrocyte counts were noted; these averaged 5,500,000 per cubic millimeter. The 20 blood smears examined revealed the presence of one or more megakaryocytes (Fig. 8) in 5 instances.

Comment

These studies would appear to throw new light on many concepts presently held concerning megakaryocytes in general and the pulmonary megakaryocytes in particular. Thus, histochemical evidence is added to morphological evidence that these cells long presumed to be megakaryocytes, occasionally seen in the spleen, liver, and lymph nodes and, as demonstrated here, seen routinely in the lungs are identical with the megakaryocytes of the bone marrow. These studies also appear to indicate that the presence of megakaryocytes in the lung capillaries is not a phenomenon of adult mammalians, as stated by Wintrobe, but may be noted at all ages, beginning in fetal life. It would also appear that the presence

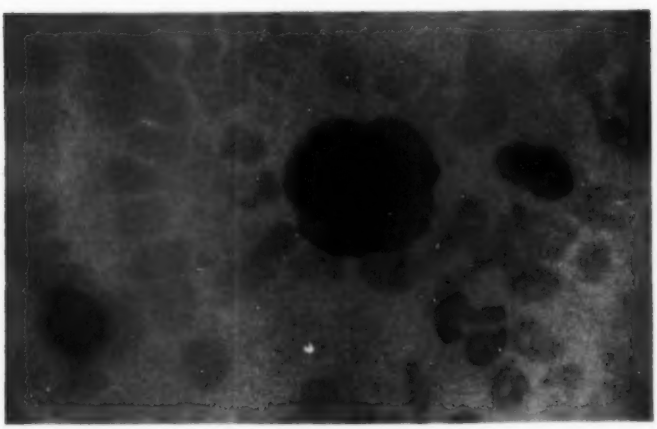


Fig. 8.—Megakaryocyte in a blood smear made from the "buffy coat" of cubital venous blood. Oil immersion; reduced about 15% from mag. × 1150.

of megakaryocytes in the lungs is not a phenomenon of disease but may also be noted in all normal mammals.

The impression gained from these studies would appear to indicate that pulmonary megakaryocytes are not "effete" cells, as they have been designated, but appear to break up in the pulmonary capillary bed into cell fragments or platelets. The megakaryocytes usually found in the bone marrow appear to be transported to the lungs via the venous circulation. This is supported by the frequent observance of the megakaryocytes as demonstrated above in the cubital venous blood. One must therefore assume that they are transported via the right side of the heart to the pulmonary vascular bed. This adds support to the concept of first Aschoff and later Goroncy of megakaryocyte embolization. From the previously described histologic appearance, the impression is gained that the megakaryocytes are broken asunder by their division into the anastomosing capillaries. The apparent induced disappearance of the megakaryocytes from the pulmonary capillaries in rabbits given epinephrine stimulation would appear to indicate that they are there as transient cells. However, the histomorphologic evidence coupled with the epinephrine-induced disappearance of the pulmonary megakaryocytes and the concomitant significant rise in platelets in the peripheral blood tend to support the suggestion of Howell and Donahue that the pulmonary capillary bed may be a site of break-up of the megakaryocytes into platelets. No evidence is obtained to negate the original observation of Wright that the bone marrow sinusoids may also be a site of platelet formation.

Evidence has also been obtained that larger numbers of megakaryocytes may be present in the lungs with greater frequency in certain disease states. These are in order of greatest incidence the postoperative and infectious states, thromboembolic disease, and severe anemias.

Summary and Conclusions

Pulmonary megakaryocytes may be observed in all mammalians routinely. They may be observed in all humans as early as in five-month fetuses and all through life in both normal and diseased persons.

The cells assumed on morphologic evidence to be megakaryocytes as seen in the lungs, liver, spleen, and lymph nodes give an identical histochemical reaction for polysaccharides to that of bone marrow megakaryocytes.

Evidence has been obtained which appears to indicate that megakaryocytes are transported by means of the venous circulation from the bone marrow to the pulmonary capillary bed, where by filtering through the vessels and their anastomoses they may be broken up to form platelets. This would appear to indicate that the pulmonary capillary bed may be a significant site of platelet formation from megakaryocytes.

Evidence has also been obtained that larger numbers of megakaryocytes appear more frequently in the pulmonary vascular bed in the postoperative and infectious states in thromboembolic disease and in severe anemias.

Dr. Leonard J. Goss, Assistant Director and Veterinarian of the New York Zoological Society, made all animal lung sections, except those of the rabbits and the squirrel, available to us for study. This publication has been made possible in part by Mr. Clifford T. Weihman, president of the Mount Vernon Hospital.

Department of Pathology, Mount Vernon Hospital.

REFERENCES

1. Cooper, T.; Stickney, J. M.; Pease, G. L., and Bennett, W. A.: Thrombotic Thrombocytopenic Purpura: Confirmation of Clinical Diagnosis by Bone Marrow Aspiration, Am. J. Med. 13:374-383, 1952.

2. Fitzgerald, P. J.; Auerbach, O., and Frame, E.: Thrombocytic Acroangiothrombosis, Blood 2: 519-541, 1947.

3. Singer, K.; Bornstein, F. P., and Wile, S. A.: Thrombotic Thrombocytopenic Purpura, Blood 2: 542-554, 1947.

4. Singer, K.; Motulsky, A. G., and Shanberge, J. N.: Thrombotic Thrombocytopenic Purpura: Studies on Hemolytic Syndrome in This Disease, Blood 5:434-448, 1950.

5. Barondess, J. A.: Thrombotic Thrombocytopenic Purpura: Review of Literature and Report of 3 Cases, Am. J. Med. 13:294-310, 1952.

6. Vassar, P. S., and Spain, D. M.: Platelet Thrombosis Syndrome, Circulation 8:664 671, 1953.

7. Wile, S. A., and Sturgeon, P.: Thrombotic Thrombocytopenic Purpura: Review of the Subject with a Report of 3 Cases in Children, Pediatrics 17:882-896, 1956.

8. Sharnoff, J. G.: Thrombotic Thrombocytopenic Purpura, Am. J. Med. 23:740-747, 1957.

9. Aschoff, L.: Über capilläre Embolie von riesenkernhaltigen Zellen, Arch. Path. Anat. 134: 11-25, 1893.

10. Goroncy, C.: Über Knochenmarksriesenzellembolie im grossen Kreislauf, Arch. path. Anat. 249:357-367, 1924.

11. Brill, R., and Halpern, M. M.: The Frequency of Megakaryocytes in Autopsy Sections, Blood 3:286-291, 1948.

12. Seebach, L. M., and Kernohan, J. W.: Megakaryocytes in Pulmonary Capillaries, Am. J. Clin. Path. 22:646-651, 1952.

13. Howell, W. H., and Donahue, D. D.: The Production of Blood Platelets in the Lungs, J. Exper. Med. 65:177 204, 1937.

14. Jordan, H. E.: Origin and Significance of the Megakaryocytes of the Lung, Anat. Rec. 77: 91-101, 1940.

15. Wintrobe, M. M.: Clinical Hematology, Ed. 4, Philadelphia, Lea & Febiger, 1956, p. 265.

16. Wachstein, M.: Distribution of Histochemically Demonstrable Glycogen in Human Blood and Bone Marrow Cells, Blood 4:54-59, 1949.

17. Storti, E.; Perugini, S., and Soldati, M.: Cytochemical Investigations of Normal Megakaryocytes and Platelets, Acta haemat. 10:144-149, 1953.

18. Wislocki, G. B.; Bunting, H., and Dempsey, W. W.: Further Observations on the Chemical Cytology of Megakaryocytes and Other Cells of the Hemopoietic Tissue, Anat. Rec. 98:527-537, 1947.

19. Minot, G. R.: Megakaryocytes in the Peripheral Circulation, J. Exper. Med. 36:1-8, 1922.

Structure and Genesis of the Mediastinal Teratoma

KIYOSHI INADA, M.D., and AKINORI NAKANO, M.D., Okayama, Japan

Mediastinal tumors requiring surgical intervention are relatively rare. Among them, teratomas of the anterior mediastinum are the commonest, ranking second only to neurogenic tumors as reported in the western literature.[1] A review of the Japanese literature, however, shows the contrary, and it appears that teratomas of the anterior mediastinum are the commonest in Japan.

There are many theories regarding the histogenesis of mediastinal teratoma, but as yet no single opinion has been in favored acceptance among pathologists. Among the numerous studies on mediastinal teratogenesis, Schlumberger's classical work[3] is probably the most attractive and comprehensive. He postulated that teratoma of the anterior mediastinum has its origin in the faulty embryogenesis of the thymus. This paper will present the results of the histological study of 15 cases of mediastinal teratomas supporting Schlumberger's concept, since no report concerning this subject has been found in the literature after him.

Submitted for publication Jan. 6, 1958.

Instructor in Surgery (Dr. Inada); Postgraduate Student in Pathology (Dr. Nakano), Okayama University Medical School.

Materials

Fourteen specimens of mediastinal teratoma were obtained by surgery and one by autopsy. All were located in the anterior mediastinum, although some were so huge, occupying the hemithorax, that their sites of origin could not exactly be determined. The outstanding clinical symptoms encountered in the whole series are tabulated in Table 1.

As many sections as possible were examined in the histological study of each case. In 3 of 15 specimens, the whole tumor tissues were examined by the multiple-block method. The pathologic findings and number of sections examined in each case are given in Table 2. There were two malignant teratomas in this series. Thirteen of the fifteen specimens were of the cystic type. There were three specimens in which thymic tissues were attached. In another case, the growth was found connected to the thymus gland at operation.

TABLE 1.—*Clinical History of the Entire Series*

Case No.	Age, Yr.	Sex	Symptoms	Complications	Size of Tumor, Cm.	Form of Tumor
1	23	F	Chest pain	None	7.5×5.5×3.2	Cystic
2	19	M	Trichophytosis	Cystobronch. communication	3.5×5×3	Cystic
3	53	F	None	None	21×13×17	Cystic
4	63	F	Dyspnea	None	18×16×12	Cystic
5	28	F	Dyspnea	Malignant change	24×22×21	Parenchymatous
6	10	M	Cough sputum	Cystobronch. communication	5×6×5.5	Cystic
7	46	M	None	Malignant change	14×10×7.5	Cystic
8	24	F	Trichophytosis	Cystobronch. communication	16×14×10	Parenchymatous
9	28	F	Cough sputum	Cystobronch. communication	10×10×8	Cystic
10	19	M	Chest pain	None	10×9×6	Cystic
11	47	F	Chest pain	None	9×7×6	Cystic
12	21	M	Cough sputum	None	4.5×3.5×3	Cystic
13	22	M	Cough sputum	None	12×9×5	Cystic
14	25	F	Chest pain	None	7.5×5×4	Cystic
15	27	F	Cough pain	None	11.5×9×10.5	Cystic

TABLE 2.—*Structural Components in Fifteen Cases of Mediatinal Tumors*

	Ectodermal Derivatives			Mesodermal Derivatives								Entodermal Derivatives					
Case No.	Squamous Epithelium	Skin & Appendage	Nerve Tissue	Connective Tissue	Vascular Spaces	Adipose Tissue	Lymphoid Tissue	Hemopoietic Tissue	Smooth Muscle	Cartilage	Bone	Respiratory Epithelium	Intestinal Epithelium	Mucous Gland	Pancreas	Thymus	Sect. Examined, No.
1	+	+		+	+	+	+		+			+		+		+	7
2	+	+		+	+	+	+		+			+		+		+	51
3				+	+	+	+		+								8
4				+	+	+	+		+								9
5*	+	+		+	+	+	+		+	+	+	+				+	13
6	+	+		+	+	+	+		+	+		+	+	+	+	+	70
7*				+	+	+	+		+			+				+	132
8	+		+	+	+	+	+	+	+	+	+	+	+	+	+		198
9	+	+		+	+	+	+		+							+	3
10	+	+		+	+	+	+		+	+	+						4
11	+	+		+	+	+	+	+	+			+		+	+	+	2
12	+	+		+	+	+	+		+	+		+	+	+	+	+	4
13	+		+	+	+	+	+		+	+		+	+	+	+	+	6
14	+	+	+	+	+	+	+		+	+		+	+	+	+	+	9
15	+	+		+	+	+	+		+							+	12
Total	12	10	3	15	15	15	15	2	15	7	3	10	5	8	6	11	528

* Malignant.

Results of Study

Most pathologists support the view that the term dermoid cyst should be discarded and that it should be included under the term of teratoma, because tissues derived from the three germinal layers are usually found in dermoid cysts after careful histological examination. This was confirmed in the present study.

Microscopically, 10 of the 15 specimens exhibited tissues from the three germinal layers. The commonest structures were epidermal in character, with hair follicles and sweat and sebaceous glands. Cartilage was present in seven cases, and bone tissue, in three. Hematopoietic tissues were seen in two cases. Striated musculature was found in one case. Well-developed pancreatic tissues, which, according to Schlumberger, is a characteristic feature of mediastinal teratoma, were found in six cases. Residual thymic tissue was found adjacent to the capsule or mixed with the tumor tissue in the marginal areas in 11 of 15 cases, in spite of the fact that the materials under examination were of limited size in several cases (Figs. 1 and 2). In one case, thymic tissue was found to be present completely around the tumor. Usually, adipose tissue replaced the involuting

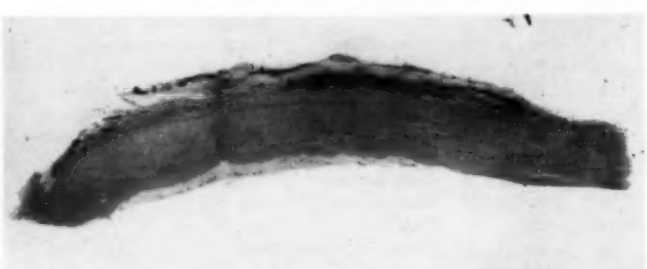


Fig. 1.—Microscopic section showing residual thymic tissue sharply defined by connective tissue from tumor tissue in the wall of cystic teratoma. Arrows indicate thymic tissue. Hematoxylin and eosin; reduced 35% from mag. ×5.

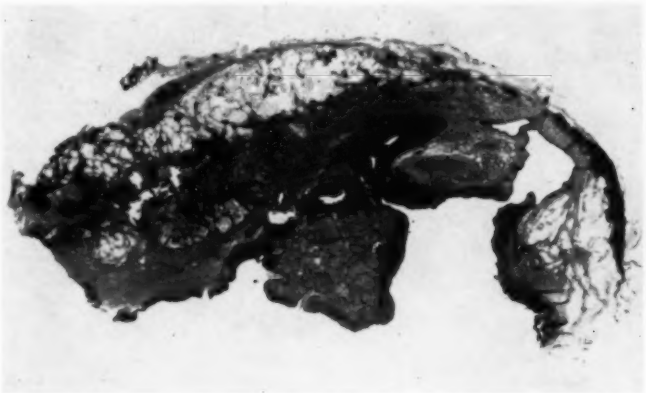


Fig. 2.—Microscopic section showing residual thymic tissue intermingled with tumor tissue in the wall of cystic teratoma. Arrows indicate thymic tissue. Hematoxylin and eosin; reduced about 35% from mag. × 4.

thymic tissue, and sometimes isolated Hassall's corpuscles, usually calcified, were seen in the hyalinized connective tissues which sharply demarcated thymic tissue from tumor tissue (Fig. 3). In two cases, there were unusual regressive changes, such as cyst formation, probably lymph cysts, and relative proliferation of large epithelial-ap-

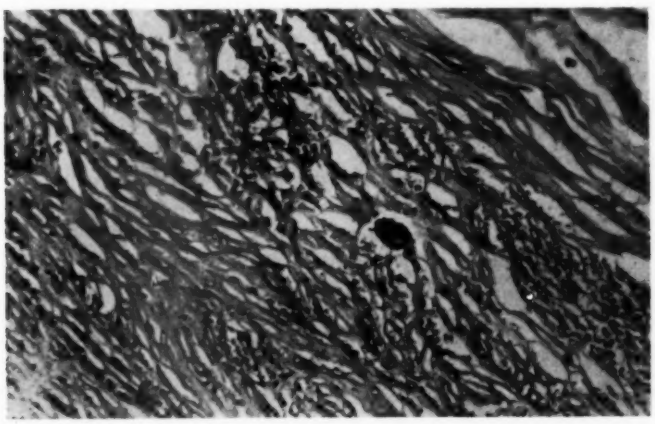


Fig. 3.—Isolated Hassall's corpuscle in the dense connective tissue of the wall of the cystic teratoma. Hematoxylin and eosin; reduced 35% from mag. × 200.

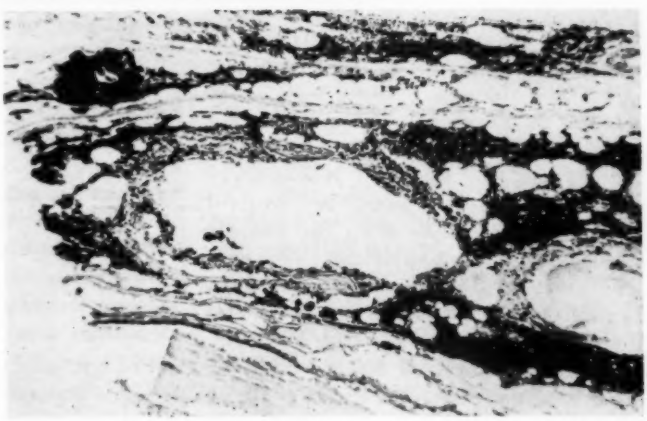


Fig. 4.—Residual thymic tissue showing regressive changes with cyst formation. Arrow indicates calcified Hassall's corpuscle. Hematoxylin and eosin; reduced 35% from mag. × 50.

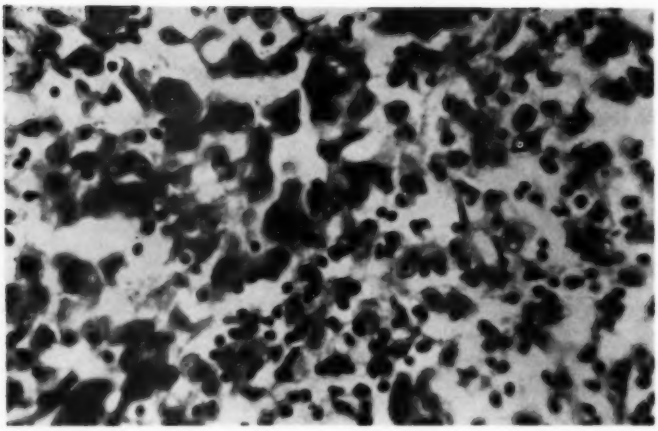


Fig. 5.—Residual thymic tissue showing proliferation of endothelial cells simulating thymoma. Hematoxylin and eosin; reduced 35% from mag. ×400.

pearing cells (Fig. 4). In one of them, proliferation of endothelial cells was so marked that the appearance gave the impression of thymoma of the lymphangioendothelial type (Fig. 5).

Histogenesis

There have been many hypotheses regarding the histogenesis of mediastinal teratomas, and an excellent review of the subject has already been published [4]; hence, another review of the literature has been omitted. Schlumberger pointed out the close relationship between the thymus gland and mediastinal teratoma. He found residual thymic tissue in the walls of anterior mediastinal teratomas in 4 of 16 specimens. He further emphasized the high incidence of pancreatic tissue found in mediastinal teratomas, as compared with its infrequent occurrence in teratomas in the gonads and other sites. In view of these findings, he ascribed the origin of anterior mediastinal teratomas to the faulty embryogenesis of the thymus and suggested that anterior mediastinal teratoma should be identified as teratoma of the thymus. The intimate relationship of mediastinal teratoma with the thymus gland has been frequently noted by surgeons and pathologists before Schlumberger, and some cases of dermoid cyst developing in the thymus have been described in the literature.[5-9] However, the importance of the study of the thymus in cases of mediastinal teratoma is not always appreciated. It is surprising that some authors reporting large numbers of mediastinal teratomas have not noted teratomas attached to or arising in the thymus, as Webb [9] pointed out.[10-12] Ringertz and Lidholm [13] recently stated that normal thymic tissue was found adjacent to the capsules in 6 of the 14 benign teratomas of the mediastinum and further stated that this phenomenon would have been noted more frequently had histological studies been made in all of the tumors *in toto*. In this series, residual thymic tissues were found in the marginal areas of the specimens in 11 of 15 cases. It is clearly seen that thymic tissue was compressed by tumor tissue, and in some cases tumor tissue and thymic tissue were not sharply defined but were intermixed with each other, indicating that they are not merely located adjacently. In one case the tumor was completely encapsulated by thymic tissue. This observation of thymic tissue in mediastinal teratoma makes it clear why mediastinal teratoma arise almost exclusively in the anterior mediastinum. It is conceivable that the thymus gland might be misplaced sometime during embryonal development into the posterior mediastinum in the case of posterior mediastinal teratoma.

The site of development of mediastinal teratoma was thus clarified. However, the

origin of the tissue is still a controversial problem. Schlumberger postulated that the ectodermal inclusions together with the degeneration of Hassall's corpuscles may initiate production of tridermal tumors. Recently, Friedman [14] made a comparative study of the histogenesis of mediastinal and gonadal teratoma by the multiple-block method. He ascribed the pluripotent germ cell to be the origin of mediastinal teratoma, and he gave a brief schema regarding tumors of germ-cell origin adaptable to gonadal and extragonadal teratoma but not to sacral teratoma (Fig. 6). The term

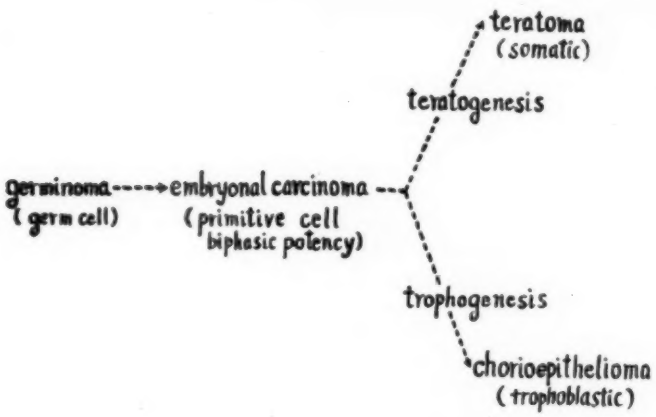


Fig. 6.—Diagram postulating development of tumors of the germinal origin. Reproduced with permission from Friedman, N. B.: *Cancer* 4: 265, 1951.

germinoma was preferred by Friedman for seminoma of the testes, for dysgerminoma of the ovary, and for identical conditions which occur extragenitally. Schlumberger,[3] however, doubts Friedman's hypothesis, though he concedes that teratoma of the gonads arises from pluripotent germ cells normally present in these organs. Since Friedman's report, evidences supporting his concept have been accumulating, although there have been reported before in the literature some cases of unquestionable primary choriocarcinoma of the mediastinum.[15-17] Pugsley and Carleton [18] reported a case of teratocarcinoma of the mediastinum which was shown to contain a nodule of germinoma and residual thymic tissue and a case of choriocarcinoma of the thymus. Woolner et al.[19] reported two cases of pure seminoma (germinoma) and a case of malignant teratoma of the anterior mediastinum in which a portion of germinoma was found on section after multiple blocks. They emphasized that, based on clinical observations, their cases were all apparently primary in the mediastinum, though pathologic examination of the testes was performed in none of them.

Iverson,[20] attempting a reclassification of the thymic neoplasms, reexamined 50 cases diagnosed as thymic growth during the two-year period from 1952 to 1954 at the Armed Forces Institute of Pathology and recognized that 8 cases previously diagnosed as the thymic carcinoma were all seminomatous tumors. He also found definite inclusion of teratomatous elements in 3 of 11 cases. He supported Friedman's view, although he considered the possibility of either the thymus or the mediastinum as the site of origin. Effler and McCormack [21] stated that they found 2 cases of anterior mediastinal seminomas among 27 thymic growths. However, they did not give any details. We,[22] using the multiple-block method, also found a case of pure germinoma of the anterior mediastinum which was considered to have developed in the thymus (Fig. 7). The patient, then 23 years old, is well four and one-half years after surgical extirpation and

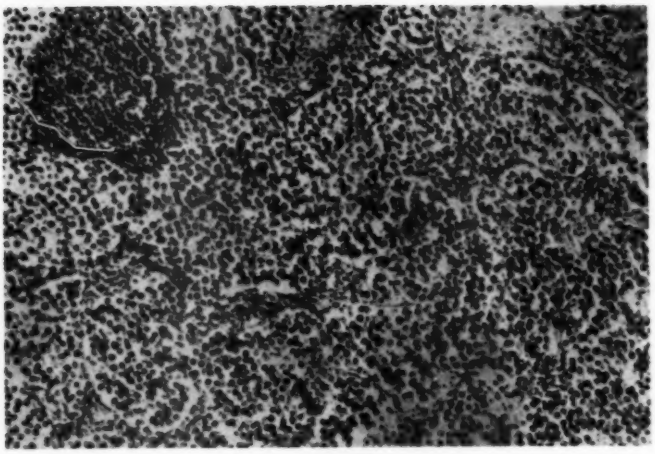


Fig. 7.—Microscopic section showing typical seminomatous tissue. Hematoxylin and eosin; reduced 35% from mag. × 100.

without evidence of metastases or abnormal findings in the testes. These cases seem to indicate that primordial germ cells might be misplaced in the mediastinum, probably in the thymus per se, and occasionally may give rise to the entire spectrum of germinal tumors. However, it is difficult to prove whether all these tumors actually represent neoplasms of primitive germ cells, though morphologically no difference between them is recognizable. However, it is hardly understandable with this theory why these tumors occur almost exclusively in young males (Table 3) and why the incidence is very small compared to the large numbers of mediastinal teratomas, although extensive histological studies have intentionally not been made in most of the reported mediastinal teratomas. These cases become significant as some of them tend to support and some seem to contradict the various hypotheses, thereby relegating the etiology of mediastinal teratoma into the realm of speculation. Further study of a large series is required.

It was clearly shown in this study that mediastinal teratoma can undoubtedly develop in the thymus. However, the question of origin of the tumor tissue still remains unanswered.

Summary

Extensive histological studies were made on 15 specimens of mediastinal teratoma, all located in the anterior mediastinum. Residual thymic tissue was found in 11 out

TABLE 3.—*Collected Cases of Mediastinal Tumors of Germinal Origin*

Author	Age, Yr.	Sex	Histologic Findings	Findings in Gonads	Remarks
Arendt	20	M	Chorioepithelioma	Atrophy marked; neoplasm (—)	Autopsy
Kantrowitz	22	M	Teratoma+chorioepithelioma	Neoplasm (—); scar (—)	Autopsy
Pugsley & Carleton	16	M	Teratoma+germinoma	Neoplasm (—); scar (—)	Autopsy
Pugsley & Carleton	37	M	Chorioepithelioma (thymus)	Neoplasm (—); scar (—)	Autopsy
Woolner et al.	31	M	Germinoma	No abnormality (clinically)	Well 3 yr. after op.
Woolner et al.	24	M	Germinoma	No abnormality (clinically)	X-ray therapy
Woolner et al.	23	M	Teratoma+germinoma	No abnormality (clinically)	Died 2.5 mo. after op.
Iverson	24 *	M †	Germinoma (8 cases) germinoma+teratomatous element (3 cases)		
Inada & Nakano	23	M	Germinoma	No abnormality (clinically)	Well 4.5 yr. after op.
Taniguchi	19	M	Germinoma	Neoplasms (—)	Autopsy

* Average of 9 cases.
† 9 of 11 cases.

of 15 specimens. In one case, thymic tissue was found to be present, completely surrounding the tumor. These findings apparently support Schlumberger's hypothesis of thymic teratogenesis. A brief review of primary seminomatous tumor (germinoma) of the mediastinum is given and Friedman's hypothesis of extragonadal germ-cell origin of mediastinal teratoma is discussed.

Department of Surgery, Okayama University Medical School.

REFERENCES

1. Lindskog, G. E., and Liebow, A. A.: Thoracic Surgery and Related Pathology, New York, Appleton-Century-Crofts, Inc., 1953, p. 509.

2. Katsura, J.; Kasai, M., and Kikuchi, H.: Mediastinal Tumors: Statistical Study in Japan, J. Jap. A. Thoracic Surg. 5:285-286, 1957.

3. Schlumberger, H. G.: Teratoma of the Anterior Mediastinum in the Group of Military Age: A Study of 16 Cases, and a Review of Theories of Genesis, Arch. Path. 41:398-444, 1946.

4. Willis, R. A.: Teratomas, in Atlas of Tumor Pathology, Subcommittee on Oncology of the Committee on Pathology of the National Research Council, Armed Forces Institute of Pathology, 1951, Sec. III, Fasc. 9.

5. Marchand, F.: Beiträge zur Kentniss der Dermoidgeschwülste, cited by Ekehorn, C.: Die Dermoidcysten des Mediastinum anticum, Arch. klin. Chir. 56:107-167, 1897.

6. Hattori, S.: Ein Fall von Mediastinalteratom und ein Beitrag zur Histogenesis desselben, Verhandl. Jap. Path. Gesellsch. 3:130-131, 1913.

7. Schmincke, A.: Pathologie des Thymus, in Henke, F., and Lubarsch, O.: Handbuch der speziellen pathologischen Anatomie und Histologie, Berlin, Julius Springer-Verlag, 1926, Vol. 8, pp. 760-809.

8. Keynes, G.: Investigation into Thymic Disease and Tumor Formation, Brit. J. Surg. 42: 449-462, 1955.

9. Webb, W. R.: Thymic Teratoma with Pedunculated, Polypoid Inclusion, Ann. Surg. 144: 915-920, 1956.

10. Harrington, S. W.: Surgical Treatment in 11 Cases of Intrathoracic Teratomas, J. Thoracic Surg. 3:50-72, 1933.

11. Blades, B.: Mediastinal Tumors, Ann. Surg. 123:749-765, 1946.

12. Sabiston, D. C., Jr., and Scott, H. W., Jr.: Primary Neoplasms and Cysts of the Mediastinum, Ann. Surg. 136:777-797, 1952.

13. Ringertz, N., and Lidholm, S. O.: Mediastinal Tumors and Cysts, J. Thoracic Surg. 31:458-487, 1956.

14. Friedman, N. B.: The Comparative Morphogenesis of Extragenital and Gonadal Teratoid Tumors, Cancer 4:265-276, 1951.

15. Arendt, J.: Das Chorionepitheliom des Mannes, Fortschr. Geb. Röntgenstrahlen 43:728-735, 1931.

16. Kantrowitz, A. R.: Extragenital Chorionepithelioma in Male, Am. J. Path. 10:531-544, 1934.

17. Wilson, S. J., and Cares, R.: Mediastinal Teratoma, Arch. Path. 39:113-116, 1945.

18. Pugsley, W. S., and Carleton, R. L.: Germinal Nature of Teratoid Tumors of the Thymus, A. M. A. Arch. Path. 56:341-347, 1953.

19. Woolner, L. B.; Jamplis, R. W., and Kirklin, J. W.: Seminoma (Germinoma) Apparently Primary in the Anterior Mediastinum, New England J. Med. 252:653-657, 1955.

20. Iverson, L.: Thymoma: A Review and Reclassification, Am. J. Path. 32:695-719, 1956.

21. Effler, D. B., and McCormack, L. J.: Thymic Neoplasms, J. Thoracic Surg. 31:60-82, 1956.

22. Inada, K., and Nakano, A.: Germinoma of the Mediastinum: A Case Report with Special Reference to Histogenesis of the Mediastinal Teratoma, to be published.

23. Taniguchi, H.: Personal communication to the authors.

Endocardial Fibroelastosis in the Adult

BURTON C. DYSON, M.D., and JOHN P. DECKER, M.D., Philadelphia

Although the normal atrial endocardium contains a prominent subendothelial fibroelastic layer, in the ventricular endocardium this is extremely thin and exhibits few or no elastic fibrils. Beneath the inconspicuous ventricular endocardium is a thin subendocardial layer of loose fibrous tissue containing blood vessels, nerves, and conduction tissue.[1,2] Normally the combined thickness of endocardium and subendocardial fibrous layer in the ventricle is less than 10μ.

Fibroelastic thickening of the ventricular endocardium has been reported in many infants dying of otherwise unexplained congestive heart failure and has been ascribed to developmental defect,[3,4] to localized anoxia,[5-8] or to an unknown metabolic defect causing myocardial weakness with secondary endocardial fibrosis.[9,10] In adults a similar-appearing ventricular endocardial fibroelastosis (parietal fibroplastic endocarditis, endocardial sclerosis, or endomyocardial necrosis) has been the subject of scattered reports, with no consensus as to etiology or pathogenesis.

Nineteen adults suffering from a variety of unrelated cardiac conditions and exhibiting ventricular endocardial sclerosis at death are reported here.

Material

Ninteen cases were selected from material available in recent autopsies to demonstrate the presence of ventricular endocardial fibroelastosis in a variety of cardiac disease conditions. The cases were divided into five groups: (*a*) idiopathic cardiac hypertrophy, (*b*) miscellaneous cardiac disease, (*c*) hypertensive cardiovascular disease, (*d*) arteriosclerotic heart disease, and (*e*) valvular heart disease.

(a) Idiopathic Cardiac Hypertrophy (Cases 1 to 8)

Significant coronary artery and valvular disease were absent. There was no history of hypertension or other recognized cause of cardiac hypertrophy.

CASE 1.—A 27-year-old white man had been in excellent health, doing heavy manual labor, until April, 1953, when dyspnea, orthopnea, malaise, and anorexia appeared. In several weeks he lost 30 lb. in weight. Rales were present in the right lower chest. On physical examination and fluoroscopy the heart was found to be greatly enlarged. A loud apical systolic murmur was heard. Urinalysis, hemoglobin, sedimentation rate, three blood cultures, and serologic tests for syphilis were normal or negative. Leukocyte count was 15,300, with 3% eosinophils. Electrocardiographic study indicated sinus tachycardia, occasional premature ventricular contractions, and nonspecific ST-T wave changes. Digitalization and low-salt diet were followed by improvement, which was maintained for the next year, during which the patient worked as a mechanic. In June, 1955, increasing dyspnea, orthopnea, and productive cough led to the final hospitalization. In addition to the findings noted earlier there was now a well-localized apical diastolic rumble and gallop rhythm. Edema of the ankles was moderate. On electrocardiographic study rapid auricular fibrillation was evident. Further enlargement of the heart was evident on fluoroscopy, with normal pulsation of all borders. Venous pressure was equivalent to 230 mm. of water. Treatment with digitalis, mercurial diuretics, prednisone, potassium chloride, and sedatives was followed by improvement. The course was afebrile. After nine days the patient signed a release and left the hospital. He died at home a few days later.

Autopsy was limited to examination of the heart, which weighed 790 gm. and was hypertrophied and dilated in all chambers. The endocardial surfaces of both ventricles were smooth, glistening, and streaked with gray (Fig. 1). Microscopically, increased fibrous and elastic tissue were present in the ventricular endocardium, which had an average thickness of 100μ. In scattered small areas of the left ventricle there were myocardial degeneration and fibrosis.

Submitted for publication Nov. 22, 1957.

Miss Nancy McNabb, MT (ASCP), prepared the tissue sections, and Mr. Robert S. Halvey made the photographs.

From the Ayer Clinical Laboratory of the Pennsylvania Hospital. Trainee, National Cancer Institute (Dr. Dyson).

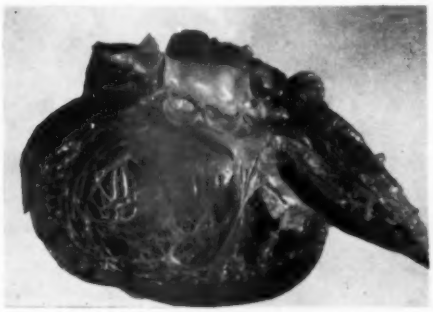


Fig. 1 (Case 1).—Man, age 27, idiopathic cardiac hypertrophy. Diffuse left ventricular translucency of slight degree. Average thickness left ventricular endocardium was 90μ.

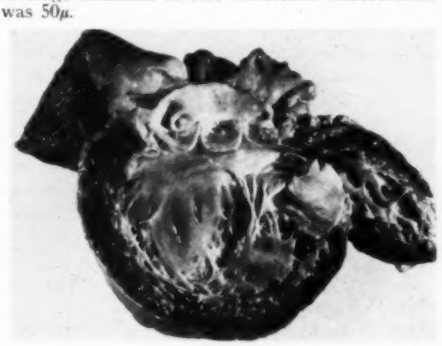
Case 2.—A 63-year-old Negro man had "never been sick a day" until 1948, when orthopnea, exertional dyspnea, productive cough, and dependent edema appeared. In the next eight years he had taken digitalis regularly and was hospitalized over 20 times for these same symptoms. Throughout this period he had been afebrile except for occasional evening temperatures of 99 to 100 F. On each examination basal pulmonary rales, varying amounts of anasarca, cardiac enlargement, auricular fibrillation, and an apical systolic murmur were noted. Pulse deficit never was more than 12 beats a minute. Urinalysis, hemoglobin, leukocyte count, sedimentation rate, serum urea nitrogen, and serologic tests for syphilis repeatedly were normal or negative in the earlier admissions. In April, 1953, venous pressure was 450 mm. of water and arm-to-tongue circulation time was 10 seconds. In December, 1954, I^{131} uptake was 16.8% (normal). Terminally the hemoglobin dropped to 9.4 gm., and serum urea nitrogen was 47 mg. Serum proteins repeatedly were close to the terminal values of 6.2 gm. total protein, 3.2 gm. albumin. On radiologic studies repeated at intervals increasing cardiac enlargement was evident which was most marked in the left ventricle. In repeated electrocardiograms auricular fibrillation and left bundle-branch block were evident. A single sudden severe episode of anterior chest pain in March, 1952, lasting several days, did not alter this electrocardiographic pattern. Terminally right bundle-branch block appeared. The patient's weight rose from 185 lb. before his illness in 1948 to 235 lb. at death. Finally, digitalis, diuretics, paracenteses, restricted sodium diet, and oxygen failed to relieve recurring anasarca, and the patient died after eight years of increasingly severe congestive heart failure.

At autopsy the heart was hypertrophied and dilated in all chambers. Organized mural thrombi were present in the right and left atrial appendages. An old frontal cerebral infarct presumably originated in an embolus from the left atrial thrombus. The ventricular myocardium was dark red in its outer half, pale red in its subendocardial half. Diffuse pearly translucency and thickening of the left ventricular endocardium was evident (Fig. 2). Fibroelastic thickening of this endocardium appeared on microscopic study (Fig. 3). Subendocardial fibrosis with inclusions of muscle was prominent. Arterial-luminal (Thebesian) vessels were prominent, surrounded with increased amounts of fibroelastic tissue. Diffuse mild myocardial fibrosis was evident in all sections. Extreme edema and congestion of all organs was present. Cardiac cirrhosis of the liver was observed.

Case 4.—A 68-year-old Negro woman had had thyroidectomy for nontoxic goiter. In the eight years before death, appetite had been poor and weight had decreased from 150 to 100 lb. In 1954, orthopnea, exertional dyspnea, and productive cough appeared and were relieved temporarily by digitalis. In January, 1956, radioactive iodine was given in treatment of refractory congestive failure, followed by loss of hair, coarseness of skin, and slowing of speech and thought. Moderate cardiac enlargement, scattered fine pulmonary rales, precordial systolic murmur, and slight edema of the legs were noted. Hemoglobin, leukocyte count, urinalysis, and serum urea were normal. Total cholesterol was 127 mg. % (normal, 130 to 240 mg. %). Congestive failure progressed, and the patient died in July, 1956.

At autopsy the ventricular endocardium, especially in the left ventricle, was smooth, white, and in some areas up to 0.1 cm. in thickness. Increased fibrous and elastic tissue accounted for this thickening. Bilateral thrombosis of leg veins or a right atrial thrombus provided sites of origin for numerous old and recent organizing pulmonary emboli with infarcts. Intimal thickening of pulmonary arterioles and pulmonary fibrosis were present.

Fig. 2 (Case 2).—Man, age 63, idiopathic hypertrophy. Diffuse left ventricular endocardial opacity. Average thickness of left ventricular endocardium was 50μ.



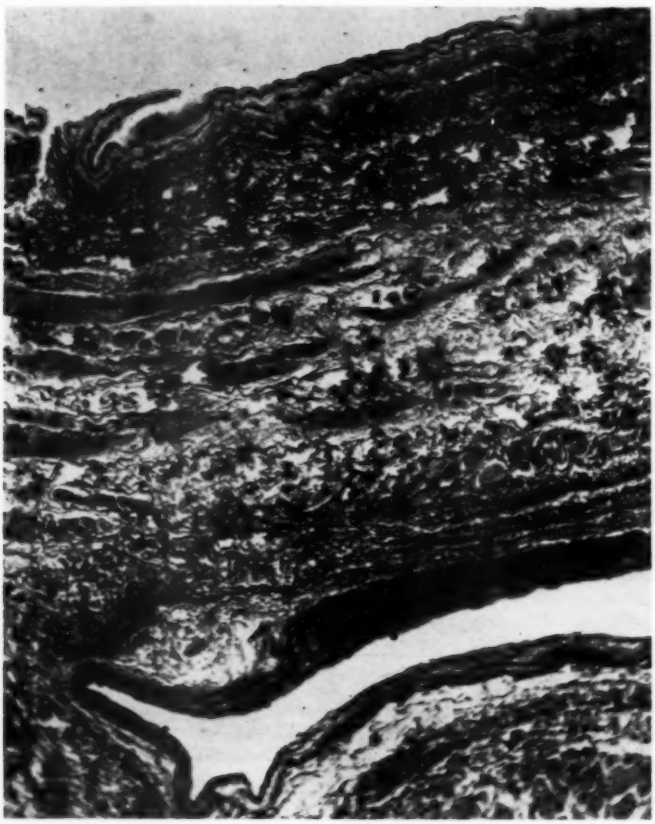


Fig. 3 (Case 2).—Marked increase of elastic fibrils within the thickened ventricular endocardium. Remnants of myocardial cells are present in the fibrous subendocardium, beneath which lies a prominent Thebesian vessel. Verhoeff-Van Gieson stain; reduced about 10% from mag. × 125.

Cor pulmonale was accompanied by passive congestion, central lobular hemorrhagic necrosis of the liver, and interstitial fibrosis of the pancreas. A healed infarct was present in the left kidney. The thyroid weighed 28 gm. and microscopically was characteristic of multinodular goiter.

Case 7.—A 56-year-old white man stated that at age 35 he had been treated for "thyroid heart disease," but in recent years no evidence of thyrotoxicosis had been present. For six years he had taken digitalis daily for congestive failure, with relief of all symptoms except occasional nocturnal paroxysmal dyspnea. Death followed embolization of the superior mesenteric artery, with acute infarction of almost the entire small bowel and right colon. At autopsy two thrombi in the left ventricle were accompanied by diffuse thickening and elastosis of the entire left ventricular endocardium.

(b) Miscellaneous (Cases 9 and 10)

Case 9 (Fiedler's Myocarditis).—A 36-year-old white man was hospitalized with acute pulmonary edema 12 months before death. Six months later a diagnosis of diabetes was made. A month later transient right hemiparesis occurred, without residual disability. Terminally there were pulmonary and peripheral edema, hepatomegaly, and several episodes of pleuritic pain, with radiographic and electrocardiographic evidence of pulmonary infarctions.

At autopsy multiple organizing mural thrombi were adherent to the mural endocardium of all four chambers, and numerous infarcts were present in kidneys, spleen, adrenal glands, lungs, pancreas, and brain. Moderate fibrosis and chronic inflammatory-cell infiltrations were present in the myocardium diffusely. In the absence of coronary artery disease of sufficient degree to produce this picture, a diagnosis of Fiedler's isolated myocarditis was made. In multiple sections from the left ventricle moderate endocardial thickening and fibrosis were present, both immediately adjacent to mural thrombi and in areas removed from such thrombi. Endocardial elastosis was moderate (Fig. 4).

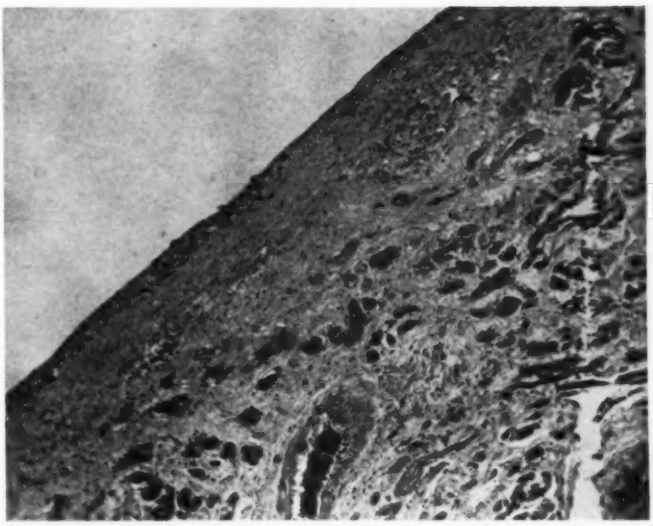


Fig. 4 (Case 9).—Man, age 36. Fiedler's myocarditis. Underlying slightly thickened endocardium is a widened subendocardial zone with chronic inflammatory cells and increased fibrous tissue. This subendocardial zone is continuous with similar myocardial interstitial tissue. Hematoxylin and eosin; reduced 30% from mag. × 120.

CASE 10.—An 85-year-old seaman died of staphylococcal bronchopneumonia. Cardiac hypertrophy with primary myocardial amyloidosis was an unexpected finding.

(c) Hypertensive Cardiovascular Disease (Cases 11, 12 and 13)

Significant narrowing of the coronary arteries and valvular diseases were absent in all cases.

CASE 12.—A 53-year-old white man who had had hypertension (blood pressure 170/100) and exertional dyspnea for three years developed progressive weakness, dyspnea, azotemia, and oliguria and died after six weeks.

At autopsy left ventricular hypertrophy and dilatation with diffuse endocardial opacity was present (Fig. 5). Microscopically, fibrous endocardial thickening and endocardial fibroelastosis were observed (Fig. 6). Pulmonary congestion, generalized visceral congestion, severe arteriolar nephrosclerosis with fibrinous pericarditis, secondary parathyroid hyperplasia, and benign prostatic hypertrophy also were noted.

CASE 13.—A 65-year-old Negro had been treated for three and one-half years for hypertension (blood pressure dropping slowly from 220/130 to 180/100). During this period were noted also weight loss, exertional dyspnea, cardiac enlargement, and an apical systolic murmur. The patient died quietly in bed after hospitalization for studies.

At autopsy coronary sclerosis of moderate degree was accompanied by a healed anterolateral myocardial infarct. Severe bullous emphysema accompanied by right heart hypertrophy was responsible for dyspnea. The left ventricular endocardium was diffusely thickened, with increase in both collagenous and elastic tissue.

(d) Arteriosclerotic Heart Disease (Cases 14, 15, 16, and 17)

Endocardial thickening was localized in the region of healed myocardial infarcts. (Figs. 7 and 8).

CASE 17.—A 47-year-old white man, four years after treatment for myocardial infarction, had a second attack of sudden severe substernal pain and died in a few minutes.

Autopsy, restricted to the thorax, revealed an enlarged heart with large anteroseptal healed myocardial infarct. No recent infarction was evident. In sections through the healed infarct (Fig. 9) endocardial fibroelastic thickening overlying a

Fig. 5 (Case 12).—Man, age 53, hypertensive heart disease. Diffuse opacity of left ventricular endocardium.

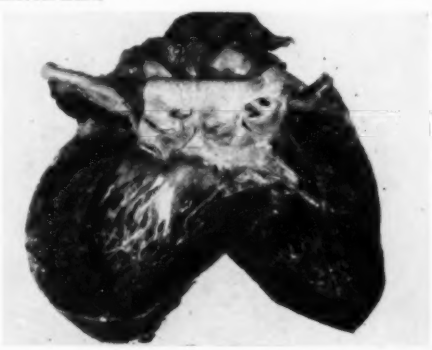


Fig. 6 (Case 12).—Elastosis in thickened ventricular endocardium. Verhoeff-Van Gieson stain; reduced 30% from mag. × 120.

layer four or five cells thick of viable myocardium covered dense fibrous scar tissue.

(e) Valvular Heart Disease (Cases 18 and 19)

The coronary arteries in these cases were without significant flaw.

Case 18.—A 71-year-old white man said to have had hypertension for 10 years developed fever and was admitted in coma, with bilateral basal rales. Blood pressure was 180/100. The serum urea nitrogen rose from 60 to 148 mg. The patient died on the third day of illness.

Fig. 7 (Case 15).—Woman, age 55, healed anteroseptal myocardial infarction with thickened opaque endocardium overlying myocardial scar.

At autopsy severe mitral stenosis with verrucal endocardiosis of the mitral valve, pulmonary fibrosis and hemosiderosis, and healed rheumatic aortic insufficiency of minor degree were present. Irregular diffuse endocardial translucency was present in the dilated left ventricle.

Case 19.—A 54-year-old Negro man two years earlier had had dyspnea and hemoptysis which led to a diagnosis of syphilitic aortic insufficiency. Response to treatment was good, with but one mild recurrence of dyspnea, until the final illness, at which time increasing dyspnea, hemoptysis, and dependent edema appeared. The patient died after several days of pulmonary edema.

At autopsy, superimposed on the right aortic valve cusp, which together, with the other two cusps was rolled, retracted, and separated, were a smooth-edged perforation and vegetations of subacute bacterial endocarditis. Endocarditic glomerulonephritis gave evidence that the valvular lesion had been present for several weeks. Nonhemolytic streptococci were cultured from the vegetations. Diffuse translucency of the endocardium in the dilated left ventricle was present.

Clinical Findings

Characteristically, the adult patient discovered at autopsy to have ventricular endocardial sclerosis clinically suffered from progressive congestive heart failure for an average duration of 23 months. No correlation between duration of congestive failure and degree of endocardial thicken-

Fig. 8 (Case 15).—Elastic fibril proliferation both in thickened endocardium and in underlying myocardial scar. Verhoeff-Van Gieson stain; reduced about 10% from mag. × 120.

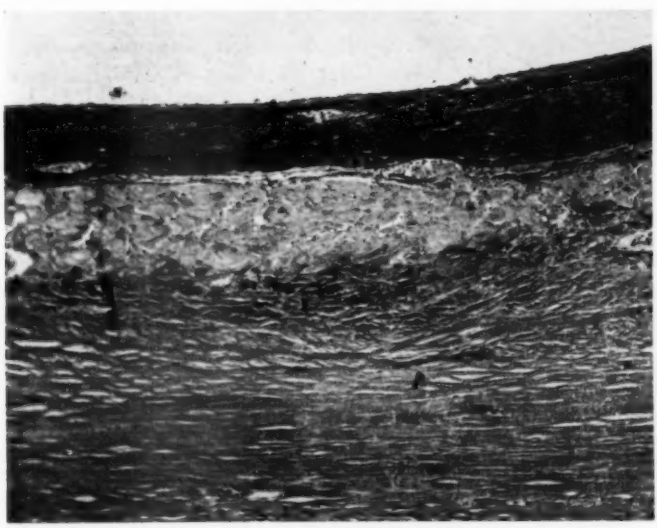


Fig. 9 (Case 17).—Marked increase in elastic fibrils in thickened endocardium overlying left ventricular healed myocardial infarct. Dense fibrous scar tissue (below) replaces myocardium except for a narrow band of viable myocardial cells beneath the thickened endocardium. Verhoeff-Van Gieson stain; reduced 30% from mag. × 120.

ing or elastosis was evident. Arrhythmias were present in eight cases. In 2 of the 18 cases in which electrocardiographic study was done, bundle-branch block was recorded (Cases 7 and 10). The average age of the group was 58 years. Males outnumbered females 16 to 3, which is approximately the ratio noted in a review of 70 cases in the literature.

In the ratio of white to Negro (10:9) and in its low socioeconomic level, this group of 19 patients paralleled the general hospital population. In six cases (Cases 1, 2, 3, 5, 7, and 17) alcoholic excess over a considerable period was reported. In Cases 3, 4, and 5 malnutrition was evident. In nutrition and use of alcohol the group was representative of the general hospital population. Although in only two of these cases (Cases 4 and 7) were peripheral embolic phenomena the immediate cause of death, evidences of embolic phenomena were present in nine of the cases. No significant correlations could be derived from the data on associated noncardiac diseases. Eosinophilia, reported in 10 of 70 cases in the literature, was absent in all of these 19 cases.

In 89 adult cases of endocardial fibroelastosis, including the 19 cases reported here and 70 other cases reported in the literature, the following clinical findings were most prominent: symptoms and signs of congestive heart failure (93%); cardiac enlargement (90%); peripheral embolic phenomena (43%); cardiac arrhythmias: regular rhythm (60%); auricular fibrillation (19%); bundle-branch block (17%), and eosinophilia (11%). Sixty-nine of these patients were men; twenty were women. The average age of the men was 42.0 years and of the women, 56.4 years.

Pathological Findings

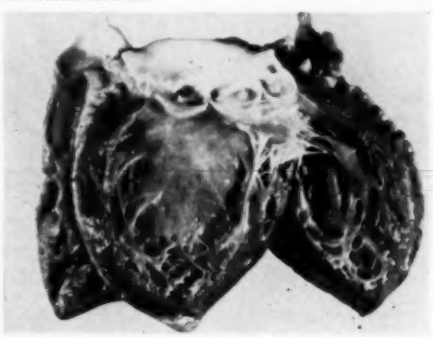
Gross.—Study of gross appearance is less satisfactory than microscopic study in appreciating the presence of definitely abnormal ventricular endocardial thickening. Compare Figures 1 and 2. Probably this is one reason the widespread occurrence of abnormal fibroelastosis in various types of heart disease has not been appreciated more generally.

In heart size no significant difference among the groups was apparent. The average heart weight was 548 gm. Hypertrophy was mostly left ventricular (except in Cases 5 and 6), but in many cases the right ventricle shared the general hypertrophy. Dilatation of all chambers was described in all except Case 15 (smallest heart studied) and Case 18 (dilation of left ventricle in aortic insufficiency).

In Group (*d*) (arteriosclerotic heart disease) localized fibroelastic thickening of the ventricular endocardium overlay myocardial scars. In all cases remaining in Groups (*a*), (*b*), (*c*), and (*e*), except Case 6, diffuse translucency or thickening of the entire left ventricular endocardium was evident but usually was of irregular degree (Figs. 1, 2, and 5). Endocardium overlying trabeculae carnae was especially thickened, although the trabeculae themselves were thinned and flattened due to cardiac dilatation (Fig. 10).

In Case 6 a single localized plaque of endocardial thickening (160μ) and elastosis was present on the midseptal portion of an otherwise normal left ventricular endocardium (Figs. 11 and 12). This may have

Fig. 10 (Case 5).—Man, age 43, idiopathic cardiac hypertrophy. Diffuse left ventricular endocardial opacity, especially over flattened and thinned trabeculae carneae.



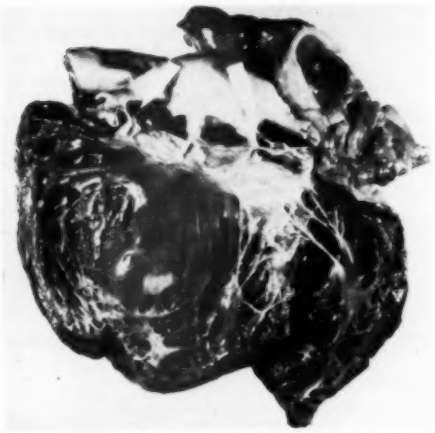


Fig. 11 (Case 6).—Man, age 58, idiopathic cardiac hypertrophy. Single localized plaque of endocardial thickening in lower interventricular septum. Fenestrations of aortic valve cusps shown.

been related to a regurgitant jet stream from several small aortic valve cusp fenestrations. Similar localized fibroelastic plaques at points of jet stream impingement on mural endocardium have been described in infant hearts with valvular lesions.[9] In an adult case recently seen in this laboratory a mural thrombus lying on the atrial endocardium above a 0.5 cm. oval fenestration in a stenotic mitral valve was formed of dense fibrous tissue with prominent elastosis.

In Group (*d*) (arteriosclerotic heart disease) mural thrombi overlay myocardial scars in three of the four cases. In the remaining 15 hearts of Groups (*a*), (*b*), (*c*), and (*e*), eight hearts exhibited mural thrombi. In five cases this involved the right atrium; in three, the left atrium; in three, the left ventricle, and in one, the right ventricle. In seven of these eight cases (excepting Case 6) peripheral embolic phenomena were noted.

Microscopic.—Sections were taken from areas showing endocardial thickening or translucency grossly and were stained with hematoxylin and eosin and by the Verhoeff-Van Gieson technique. In sections from Cases 7, 9, 11, 14, and 15, portions of mural thrombus were present. In all other cases thrombi were not present in sections. The average left ventricular endocardial thickness is noted in Table 1 in all 16 cases in which such thickening was diffuse. In the three exceptions (Cases 6, 15, and 17) the endocardial thickening was localized and is described in the text elsewhere.

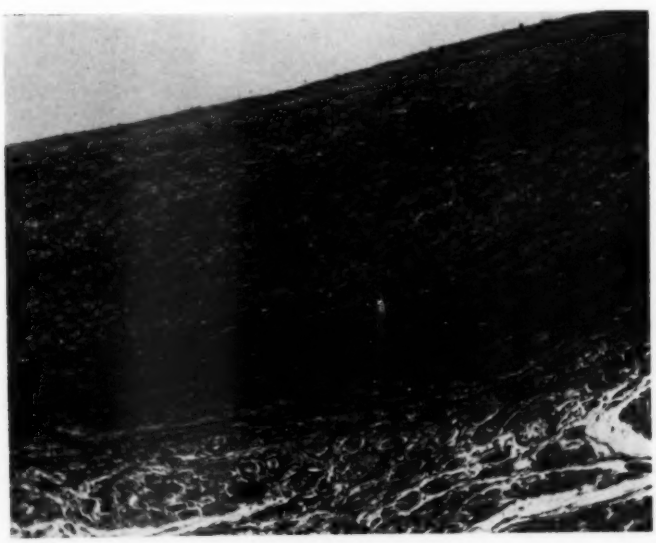


Fig. 12 (Case 6).—Marked endocardial thickening (160μ) and elastic fibril proliferation in plaque. Subendocardial zone remains normal here. Verhoeff-Van Gieson stain; reduced 30% from mag. × 120.

TABLE 1.—*Review of Nineteen Cases of Present Study*

Case	Sex	Age	Duration Congestive Failure	Heart Weight, Gm.	Endocardium Thickness, μ	Location Mural Thrombi *	Organs Infarcted	Remarks
				Group (A)—Idiopathic Cardiac Hypertrophy				
1	M	27	2 yr.	790	90	--	--	Autopsy limited to heart
2	M	63	8 yr.	780	50	RA, LA	Brain	None
3	M	59	6 mo.	510	25	RA	Lung, brain	Poorly nourished; adrenal cortical hyperplasia
4	F	68	2 yr.	560	65	RA	Lung (fatal)	None
5	M	43	5 yr.	540	85	--	--	Poorly nourished; alcoholic encephalopathy
6	M	58	9 mo.	500	15	RA, LA	--	Localized plaque endocardial thickening
7	M	56	6 yr.	690	20	LV	Mesenteric (fatal)	None
8	M	60	3 mo.	520	60	--	--	Post necrotic cirrhosis; organic brain syndrome; diabetes mellitus
				Group (B)—Miscellaneous Heart Disease				
9	M	36	2 yr.	550	50	RA, RV, LV	Multiple	Fiedlers myocarditis; diabetes mellitus
10	M	85	None	500	55	--	--	Cardiac amyloidosis
				Group (C)—Hypertensive Cardiovascular Disease				
11	M	63	18 mo.	560	25	LV	Spleen, kidney	Adrenal cortical hyperplasia
12	M	53	18 mo.	650	75	--	--	Benign prostatic hypertrophy; uremia
13	M	65	2 yr.	580	85	--	Kidney	Bullous emphysema
				Group (D)—Arteriosclerotic Heart Disease				
14	M	63	3 yr.	490	70	LV	Kidney	Diabetes mellitus
15	F	55	None	270	125	LV	--	Metastatic carcinoma of stomach
16	F	79	2 yr.	500	40	LV	--	Diabetes mellitus
17	M	47	None	450	35	--	--	Bulbous emphysema
				Group (E)—Valvular Heart Disease				
18	M	71	None	420	10	LA	Kidney	Rheumatic mitral stenosis and aortic insufficiency; B. P. 180/100
19	M	54	2½ yr.	640	25	--	--	Syphilitic aortic insufficiency & subacute bacterial endocarditis; B. P. 200/40

* RA indicates right atrium; LA, left atrium; LV, left ventricle; RV, right ventricle.

Myocardial fibrosis was marked in Group (*d*) (arteriosclerotic heart disease). In several of the cases in the other groups it was minimal or absent. No correlation between the degree of myocardial fibrosis and the degree of endocardial fibrosis, endocardial thickening, or elastosis was evident. Except in Case 9 (Fiedler's myocarditis), myocardial inflammatory infiltrates were minimal or absent.

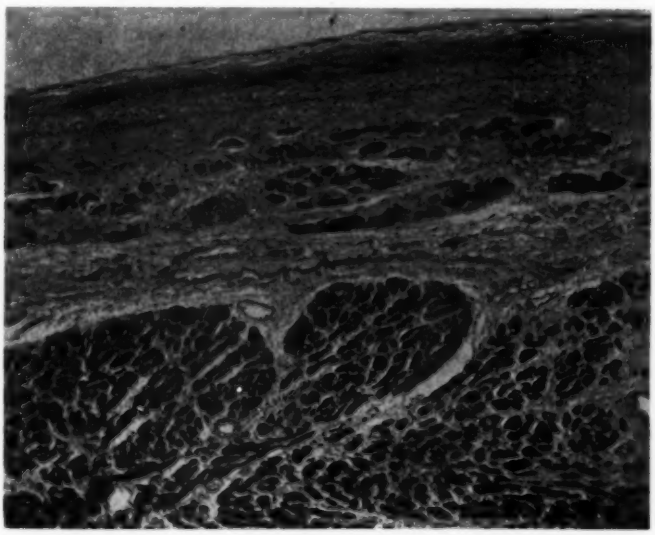


Fig. 13 (Case 5).—Remnants of muscle cells and dilated capillaries in thickened endocardium. Hematoxylin and eosin; reduced 30% from mag. × 120.

TABLE 2.—*Review of Previous Cases of Ventricular Endocardial Fibroelastosis in Adults*

Author	Case No.	Sex	Age	Associated Noncardiac Disease and Remarks
Levy & Rousselot,[11] 1933	1	M	29	....
	2	M	18	
	3	M	31	Tuberculous inguinal lymphadenitis
Loeffler,[12] 1936	1	F	45	Eosinophilia
	2	M	31	Eosinophilia
Comeau,[13] 1937	1	M	41	Epileptic attacks 1 yr.; Stokes-Adams syndrome; fibrosis & lymphocytic infiltrations in Purkinje system
	2	F	47	Hyperthyroidism 3 yr.
Mumme,[14] 1940		M	25	Disseminated sclerosis suspected; organized & recanalized emboli in most organs; B. P. 160/110; eosinophilia
Smith & Furth,[15] 1943	1	M	35	Poor diet, gastrointestinal symptoms
	2	F	35	Poor diet; gastrointestinal symptoms
	3	M	40	Poor diet; gastrointestinal symptoms
	4	M	63	Poor diet & alcoholism
	5	M	54	Poor diet & alcoholism
Egger,[16] 1944		F	49	Eosinophilia, rheumatoid arthritis 10 yr.; active mediastinal tuberculous lymphadenitis; recurring tonsillar abscesses
Roulet,[17] 1944		M	48	Eosinophilia; eosinophilic granulomata of splenic arteries; local embolic nephritis
Flynn & Mann,[18] 1946	1	M	29	Advanced pulmonary tuberculosis; inflammatory infiltrate in right ventricular & right atrial endocardium which was lined with mural thrombi
	2	M	49	14 hospitalizations in 13 yr. for congestive failure
Solomon & Leff,[19] 1947		F	75	Beefy red tongue, no sign of beriberi; cor pulmonale; mural thrombi in right heart; multiple pulmonary infarcts
Lennox,[20] 1948		M	28	Chorea age 20; anemia (Hb. 6.2 gm.); died in status asthmaticus; eosinophilia; inflammation of right ventricular endocardium; heart grossly normal
Feinberg & Holzman,[21] 1951		M	28	Repeated attacks malaria followed by congestive failure; stenosis of abdominal aorta
Gray,[22] 1951	1	M	40	Eosinophilia; jaundice following yellow fever immunization; malaria; loa loa
	2	M	36	Eosinophilia; loa loa; spastic tetraplegia with congestive failure; endocardial granulation with mural thrombi in both ventricles
Gancher,[23] 1951		M	32	....
McKusick & Cochran,[24] 1952		F	43	Subendocardial granulation tissue
Higginson et al.,[25] 1952		M	35	12 autopsied cases with chronic malnutrition, primary liver injury, cardiac dilatation & hypertrophy, normal myocardium; in these 5 cases ventricular mural thrombi were accompanied by fibrous endocardial thickening adjacent to the thrombi
		F	68	
		M	23	
		F	21	
		F	30	
Hughes & Smith,[26] 1953		M	54	2 old posterior infarcts; 1 recent anteroseptal infarct; mural thrombi in all chambers; pulmonary infarcts
Becker et al.,[27] 1953				40 autopsied cases of "all ages," "equal distribution" as to sex, with necrosis or fibrosis of parietal endomyocardium related to overlying mural thrombi; widespread arteriolar fibrinoid degeneration & necrosis; eosinophilia inconstant
McNicol et al.,[28] 1953	1	M	34	Cervical lymphnodectomy age 12, diagnosis unknown; duodenal ulcer age 27
	2	M	27	....
Ball et al.,[29] 1954 & Davies & Ball,[29] 1955	15 males, ages 18–55 (average 32.5)			Africans from Uganda; tricuspid & mitral valve defects; congestive failure; mural thrombi with embolization
		F	35	
		F	40	
		F	29	
Thomas et al.[31] 1954	10 males, ages 16–71 (average, 42.6)			
		F	49	
		F	50	
Hoffman et al.[32] 1955		M	23	Eosinophilia; hemorrhagic appendix removed 10 mo. before onset congestive failure; localized endocardial thickening right ventricle with shortening tricuspid cordae; mitral stenosis; aortic valve vegetations
Clark et al.[33] 1956		M	35	Eosinophilia; recurrent malaria
Horsley & Prec,[34] 1956		F	54	Cancerous argentiffinoma intestine; tricuspid valve leaflets thickened, pulmonic stenosis; right heart hypertrophied; fibrous plaques on right ventricular endocardium
Guraib & Rigdon,[35] 1956		M	38	Irradiated leukemia cutis; leukemic infiltrations in heart
Popper et al.,[36] 1956		M	35	Tricuspid valve ring stenosis
Stemmerman,[37] 1957		F	29	Hyperthyroidism in 1950
Wiener & Knights,[38] 1957		M	45	Eosinophilia; endocardial inflammatory infiltrate; allergic signs & symptoms
Penfold,[39] 1957		M	67	Benign prostatic hypertrophy with transient B. P. 160/110; later B. P. 110/80

The endothelium exhibited no abnormalities. In general, the endocardium and subendocardial layer were thickened to about the same degree. Increase in elastic fibrils did not always parallel the increase in thickness of the endocardium and in general was greater where the endocardium and subendocardium were not greatly increased in thickness.

A few scattered lymphocytes and histiocytes were present in the subendocardial layer of all hearts having myocardial scars —Cases 11, 13, 14, 15, 16, and 17 in Groups (*c*) and (*d*), in Case 9 (Fiedler's myocarditis), and in Case 19 (subacute bacterial endocarditis). In no case was significant inflammatory infiltrate present in the endocardium. Inclusions of muscle were pres-

ent in the thickened endocardium in half the cases (Fig. 13).

Except as noted above, no significant microscopic differences among the five groups, Groups (*a*) to (*e*), were noted in the fibroelastic thickening of the ventricular endocardium in these diverse types of heart disease. The average combined width of ventricular subendocardial fibrosis and the endocardium in these 19 cases was 53μ (normal, less than 10μ). Moderate to marked examples of elastic fibril increase were present in each of the five groups (Table 2).

Pathogenesis

While extreme thickening of the ventricular endocardium is encountered uncommonly in the adult, moderate thickening definitely beyond the normal 10μ is not infrequent and is found in a wide variety of conditions. When localized in the region of healed myocardial infarcts, as in Cases 14, 15, 16, and 17 (Figs. 7 and 8), obviously the endocardial changes are secondary to underlying myocardial lesions. In almost all other cases, however, precise understanding of the origin and pathogenesis is impossible in the individual case.

No constant cardiovascular phenomena are associated with ventricular endocardial fibroelastosis. Occasionally in hypertensive heart disease[18] endocardial thickening is present as in Cases 11, 12, 13, and 18 (Figs. 5 and 6); but in most cases of hypertensive heart disease endocardial thickening is not present, and in almost all the reported cases of endocardial fibroelastosis in adults there was normal blood pressure. Congestive heart failure is a chief clinical finding in these patients, yet in most cases of congestive failure there is no endocardial thickening.[31] The same may be said for cardiac hypertrophy and dilatation. Valvular defects have been noted in association with parietal endocardial lesions,[9,29,30,32,34] and in Cases 18 and 19 valvular lesions were prominent. However, in most cases of valvular heart disease the mural endocardium is normal. There is no association with myocardial fibrosis. Neither in the cases reviewed from the literature nor in these 19 cases were significant associations with noncardiac diseases established. Alcoholism and malnutrition[15,25] have been suggested as being pathogenetic and were present in a few of these cases—but many more here and in the literature lacked these disorders. The same might be said of thyroid disease[13,37] noted in Cases 4 and 7, tuberculosis,[11,16,18,28] and various tropical infectious diseases.[21,22,23] In many cases multiple possible causes are inextricably interrelated, as, for example, multiple pulmonary infarcts causing pulmonary hypertension and cor pulmonale, but with malnutrition simultaneously present, in Case 4, and the similar case of Solomon and Leff.[19] In Cases 11 and 13 hypertension seemed most important as a cause of heart disease, but small healed anterolateral myocardial infarcts also were present. In Cases 18 and 19 valvular lesions seemed primary in causing heart disease, but hypertension also was present.

Several writers have subdivided the cases grouped together in Table 2 on the basis of eosinophilia,[12,20,22,38,40] endomyocardial inflammatory infiltrates,[31] or elastosis.[30] Eosinophilia and endomyocardial infiltrates were lacking, and elastosis was present in all of our 19 cases. However, Loeffler[40] has indicated that transient eosinophilia may be missed unless sought frequently and persistently. Moreover, since our knowledge of endocardial fibroelastosis is based entirely on autopsy studies and therefore limited to observation of one moment in the course of each case, it is difficult to be sure that the apparent diversity of histopathologic detail reported in the 89 cases here tabulated may not represent differing stages in the same disease process. For these reasons there are included here all well-documented adult cases of ventricular endocardial fibroelastic thickening met in

surveying the literature. The variety of disease states in which this endocardial lesion is reported is striking.

Morphologic resemblances between adult and infantile cases of ventricular endocardial thickening do not indicate a congenital origin of the adult lesion. Indeed, even infantile endocardial fibroelastosis is probably acquired [9,10,41] as a compensatory endocardial response to underlying myocardial weakness of diverse causes. Still [42] has shown that surface layers of infantile endocardial thickening are formed of fibrin, presumably deposited by the blood stream, while deeper layers also contain elastic fibrils. Bunting [43] has shown that elastic tissue appears in fibrous pleural, pericardial, and upper abdominal adhesions and in myocardial scars—all being scar tissue subject to rhythmic and fluctuating strains. Black-Schaffer [41] has suggested that the elastic fibril proliferation in infantile endocardial fibroelastosis probably results from the rhythmic and fluctuating strains to which endocardium is subjected when the underlying myocardium is weakened from any of a variety of causes. Hass [2] has reported that the development of elastic tissue is a slow process, which may explain the variations in the amount of elastic proliferation found in these endocardial lesions.

Endocardial fibroelastosis localized over areas of healed myocardial infarcts obviously is due to the underlying myocardial defect (Figs. 7 and 8). That such endocardial thickening is not produced by local anoxia, as is suggested many times,[5-8] is indicated in certain cases by the preservation of a thin layer of viable myocardial cells between the thickened endocardium and the dense fibrous myocardial scar [41] (Fig. 9).

Endocardial fibroelastic thickening is not constantly associated with cardiac hypertrophy, dilatation, congestive failure, vascular hypertension, valvular defects, myocardial scars, general malnutrition, metabolic diseases, infections, or endomyocardial inflammation. What combinations of these or other factors lead to endocardial fibrosis is not known, nor is it known by what intermediate steps the endocardial fibrosis and elastosis are induced. It seems unlikely that a congenital hyperplasia is involved or that local anoxia is essential. Probably the endocardium becomes thickened by the accretion of fibrin from the bloodstream, with subsequent development of elastic fibrils in the ever-moving and slowly organizing lesion. This accretion can result from mechanical weakness of the underlying myocardium or from impingement of abnormal currents of blood in the ventricle.

The similarities to intimal lesions of arteriosclerosis overlying thinned and damaged arterial wall are obvious. Indeed, omitting elastic fibril proliferation (produced by movements present in the heart but absent in the arteries) and cholesterol deposition (perhaps a secondary event in the arterial lesions), a similarity in the histologic appearance of fibrous thickening of the ventricular endocardium and intimal lesions of arteriosclerosis is evident. Embryologically and functionally the heart is a specialized muscular artery, and so similarities in lesions of endocardium and arterial intima should not be surprising. If this analysis is correct, the proper name of the endocardial lesion discussed here would be "endocardial sclerosis," corresponding to the analogous arteriosclerosis.

Summary

In 19 adults autopsied in recent years fibroelastic thickening of the ventricular endocardium was encountered in the following types of heart disease: idiopathic cardiac hypertrophy (8 cases), miscellaneous (2 cases), hypertensive (3 cases), arteriosclerotic (4 cases), and valvular (2 cases).

Endocardial gross and microscopic lesions were similar in all five groups.

The gross appearance of endocardial fibroelastosis is less constant and striking than is the microscopic lesion. This may explain why the widespread occurrence of

this lesion in various types of heart disease has not been appreciated more generally.

Progressive congestive heart failure was the outstanding symptom in all groups, averaging 23 months in duration. Cardiac mural thrombosis with peripheral embolic phenomena was present in nine cases and was the proximate cause of death in two cases.

In these 19 cases, as in 70 adult cases noted in the literature, males outnumbered females by more than a 3:1 ratio.

Neither in these 19 cases nor in 70 adult cases noted in the literature was any specific cardiac or noncardiac cause evident for the endocardial lesion.

Fibrous thickening of the endocardium in the adult is probably a compensatory endocardial response to underlying myocardial weakness resulting from diverse causes. Elastosis characteristically is found in scars subjected to fluctuating and rhythmic stresses.

"Endocardial sclerosis" in the specialized muscular artery called the heart, corresponding to arteriosclerosis in the other arteries, is suggested as a proper term for this lesion, which is commoner in the adult than has been appreciated.

Ayer Clinical Laboratory, Pennsylvania Hospital, 8th & Spruce Sts. (7).

REFERENCES

1. Maximow, A. A., and Bloom, W.: A Textbook of Histology, Philadelphia, Ed. 4, W. B. Saunders Company, 1942.

2. Hass, G. M.: Elastic Tissue, Arch. Path. 27: 334 and 583, 1939.

3. Gross, P.: Concept of Fetal Endocarditis: General Review with Report of Illustrative Case, Arch. Path. 31:163-177, 1941.

4. Prior, J. T., and Wyatt, T. C.: Endocardial Fibro-Elastosis: Study of 8 Cases, Am. J. Path. 26:969-987, 1950.

5. Craig, J. M.: Congenital Endocardial Sclerosis, Bull. Internat. A. M. Museums 30:15-67, 1949.

6. Johnson, F. R.: Anoxia as a Cause of Endocardial Fibroelastosis in Infancy, A. M. A. Arch. Path. 54:237-247, 1952.

7. Weinberg, T., and Himmelfarb, A. J.: Endocardial Fibroelastosis (So-Called Fetal Endocarditis): Report of 2 Cases Occurring in Siblings, Bull. Johns Hopkins Hosp. 72:299-306, 1943.

8. Levi, D., and Falk, W.: Endocardial Fibroelastosis, Acta med. orient. 15:1-9, 1956.

9. Anderson, D. H., and Kelly, J.: Endocardial Fibroelastosis: 1. Endocardial Fibroelastosis Associated with Congenital Malformations of the Heart, Pediatrics 18:513-538, 1956.

10. Kelly, J., and Anderson, D. H.: Congenital Endocardial Fibroelastosis: 2. A Clinical and Pathological Investigation of Those Cases Without Associated Cardiac Malformations, Including Report of 2 Familial Instances, Pediatrics 18:539-555, 1956.

11. Levy, R. L., and Rousselot, L. M.: Cardiac Hypertrophy of Unknown Etiology in Young Adults: Clinical and Pathological Study of 3 Cases, Am. Heart J. 9:178-195, 1933.

12. Loeffler, W.: Endocarditis parietalis fibroplastica mit Bluteosinophilie: Ein eigenartiges Krankheitsbild, Schweiz. med. Wchnschr. 66:817-820, 1936.

13. Comeau, W. J.: Diffuse Parietal Endocardial Sclerosis: Review of Literature and Report of 2 Cases, Am. J. Path. 13:277-288, 1937.

14. Mumme, C.: Zur Klinik und Pathologie der Endokarditis und Aortitis fibroplastica sowie Thromboendarteritis obliterans mit hochgradiger Eosinophilie im Blut, Knochenmark und in den Organen, Ztschr. klin. Med. 138:22-50, 1940.

15. Smith, J. J., and Furth, J.: Fibrosis of Endocardium and Myocardium with Mural Thrombosis: Notes on Its Relation to Isolated (Fiedler's) Myocarditis and to Beriberi Heart, Arch. Int. Med. 71:602-619, 1943.

16. Egger, P.: Endocarditis obliterans: Ihre Beziehungen zur Endocarditis parietalis fibroplastica mit Bluteosinophilie und zum Morbus Buerger, Schweitz. Z. Path. 7:237-250, 1944.

17. Roulet, F.: Beitrag zur Kenntnis der "Endocarditis parietalis fibroplastica mit Bluteosinophilie (Loeffler)," Schweiz. med. Wchnschr. 74:427-430, 1944.

18. Flynn, J. E., and Mann, F. D.: Presence and Pathogenesis of Endocardial and Subendocardial Degeneration, Mural Thrombi, and Thromboses of Thebesian Veins in Cardiac Failure from Causes Other than Myocardial Infarction, Am. Heart J. 31:757-768, 1946.

19. Solomon, H. A., and Leff, W. A.: Acute Exacerbation of a Chronic Cor Pulmonale, New York J. Med. 47:609-613, 1947.

20. Lennox, B.: Acute Parietal Endocarditis in a Case of Status Asthmaticus: Possible Early Stage of Löffler's Endocarditis Parietalis Fibroplastica with Eosinophilia, J. Bact. & Path. 60: 621-628, 1948.

21. Feinberg, R., and Holzman, D.: Primary Parietal and Valvular Endocardial Sclerosis with

Congenital Myocardial Deformity of Right Ventricle in World War II Veteran, Bull. Internat. A. M. Museums 32:34-56, 1951.

22. Gray, I. R.: Endocardial Fibrosis, Brit. Heart J. 13:387-396, 1951.

23. Gancher, R.: Cardiac Enlargement and Failure, with Endocardial Fibrosis: A Case Report, Connecticut M. J. 15:379-384, 1951.

24. McKusick, V. A., and Cochran, T. H.: Constrictive Endocarditis: Report of Case, Bull. Johns Hopkins Hosp. 90:90-97, 1952.

25. Higginson, J.; Gillanders, A. D., and Murray, J. F.: The Heart in Chronic Malnutrition, Brit. Heart J. 14:213-224, 1952.

26. Hughes, A., and Smith, G. H.: A Case of Endocardial Fibrosis Showing Heparin Resistance, Brit. Heart J. 15:450-452, 1953.

27. Becker, B. J. P.; Chatgidakis, C. B., and van Lingen, B.: Cardiovascular Collagenosis with Parietal Endocardial Thrombosis: Clinicopathologic Study of 40 Cases, Circulation 7:345-356, 1953.

28. McNicol, C.; MacMahon, H. E.; Benenson, A. S., and Winship, T.: Recurrent Parietal Thromboendocarditis, Circulation 7:497-502, 1953.

29. Ball, J. D.; Williams, A. W., and Davies, J. N. P.: Endomyocardial Fibrosis, Lancet 1:1049-1054, 1954.

30. Davies, J. N. P., and Ball, J. D.: Pathology of Endomyocardial Fibrosis in Uganda, Brit. Heart J. 17:337, 1955.

31. Thomas, W. A.; Randall, R. V.; Bland, E. F., and Castleman, B.: Endocardial Fibroelastosis: A Factor in Heart Disease of Obscure Etiology; Study of 20 Autopsied Cases, New England J. Med. 251:327-338, 1954.

32. Hoffman, F. G.; Rosenbaum, D., and Genovese, P. D.: Fibroplastic Endocarditis with Eosinophilia, Ann. Int. Med. 42:668-680, 1955.

33. Clark, G. M.; Valentine, E., and Blount, S. G., Jr.: Endocardial Fibrosis Simulating Constrictive Pericarditis, New England J. Med. 254: 349-355, 1956.

34. Horsley, D. B., and Prec, O.: Chronic Pulmonic and Tricuspid Stenosis with a Cancerous Argentiffinoma, A. M. A. Arch. Int. Med. 97:806-813, 1956.

35. Guraib, S. R., and Rigdon, R. H.: Fibroelastosis in Adults, Am. Heart J. 52:138-148, 1956.

36. Popper, H.; Kushner, D. S., and Gasul, B.: Adult Fibroelastosis with Congenital Tricuspid Stenosis, Circulation 14:412-421, 1956.

37. Stemmerman, G. A.: Idiopathic Endomyocardial Necrosis, Am. J. Med. 22:142-150, 1957.

38. Wiener, M. J., and Knights, E. M., Jr.: Löffler's Endocarditis Parietalis Fibroplastica with Eosinophilia, Am. Heart J. 53:157-161, 1957.

39. Penfold, J. B.: Endocardial Fibrosis of Unknown Origin, Lancet 1:456-457, 1957.

40. Loeffler, W.: Pathogenetic Significance of So-Called Endocarditis Parietalis Fibroplastica, Bull. schweitz, Akad. med. Wissensch. 2:287-291, 1947.

41. Black-Schaffer, B.: Infantile Endocardial Fibroelastosis: A Suggested Etiology, A. M. A. Arch. Path. 63:281-306, 1957.

42. Still, W. J. S., and Boult, E. H.: Pathogenesis of Endocardial Fibro-Elastosis, Lancet 2: 117-118, 1956.

43. Bunting, C. H.: New Formation of Elastic Tissue in Adhesions Between Serous Membranes and in Myocardial Scars, Arch. Path. 28:306-312, 1939.

On the Role of Vitamin D in Calcification

MICHAEL LAMM and W. F. NEUMAN, Ph.D., Rochester, N. Y.

The rickets syndrome resulting from a dietary deficiency of vitamin D, though well described, has never been fully explained. One of the unsettled questions concerns the failure of cartilage and osteoid (bone matrix) to mineralize properly. "The main question is whether the disturbed mineralization is due only to a decreased Ca×P quotient in serum, and consequently also in extracellular fluid, or whether there is in addition some primary disturbance in the bone-forming tissue." [1]

As long as we are unsure of the molecular and cellular mechanisms involved in the calcification process itself, the role of vitamin D in mineralization will, of course, remain unsettled. Recently, however, there has appeared new evidence that the calcification process may involve, at least in part, a catalyzed (by collagen), epitactic induction of crystals [2-8] from the body fluids which are normally supersaturated with respect to bone mineral.[3,6,9,10] From this viewpoint, it is of interest to know whether normal bone osteoid is able to induce crystal formation in solutions corresponding to rachitic serum products, Ca×P, and, conversely, whether rachitic osteoid is able to induce crystal formation in solutions corresponding to normal serum products, Ca×P. Accordingly, the following experiments were performed to test the seeding ability of thinly sectioned slices of thoroughly demineralized tibial bone ends of normal and D-deficient rats.

Similar experiments have appeared in the literature many times (compare, one of the latest [11]). With very few exceptions,[12,14] however, surviving sections (nondemineralized) have been placed in serum or serum-like solutions. To avoid the complications derived from cellular activity or from the presence of preformed mineral which, by itself, can initiate further crystal growth, only nonvital exhaustively demineralized tissue sections were employed in these experiments.

Experimental Data

Reagents.—The demineralizing solution, ethylenediamine-tetraacetic acid (Versene, EDTA), was prepared as a 0.7 M solution at pH 7.4 by the addition of KOH.

All mineralizing solutions were prepared as free of CO_2 as possible through the use of boiled distilled water and an N_2 atmosphere over all transfers. The ionic strength was in all cases 0.16, and the pH, 7.4. This was accomplished by mixing, in varying proportions to obtain the various Ca×P products, the following three solutions: Solution A. KH_2PO_4, 0.0107 M; KCl, 0.117 M; barbituric acid, 0.02 M; adjusted with CO_2-free KOH. Solution B. $CaCl_2$, 0.0056 M; KCl, 0.128 M; barbituric acid, 0.02 M; adjusted with CO_2-free KOH. Solution C. The diluting solution—KCl, 0.145 M; barbituric acid, 0.02 M, also CO_2-free and KOH-adjusted. The presence of 0.02 M barbital, in the absence of CO_2, insured good buffering. To avoid precipitation, the calcium solution was always the last reagent added to the mixture.

All solutions were stored at 4 C in stoppered Parafilm-sealed flasks to which a few crystals of thymol had been added to prevent bacterial growth.

Methods.—Thirty Rochester-Wistar strain rats, 21 days of age and of both sexes, were randomly mixed and placed on the standard high-calcium low-phosphate U. S. P. rachitogenic diet (Nutritional Biochemicals Corp., Cleveland). Food and distilled water were given ad libitum. Fifteen rats (the experimentals) received no supplements;

Submitted for publication Feb. 25, 1958.

From the Department of Pathology and the Division of Pharmacology, Department of Radiation Biology, the University of Rochester School of Medicine and Dentistry.

This paper is based on work in part supported by a National Science Foundation Summer Fellowship and in part supported under contract with the United States Atomic Energy Commission at the University of Rochester Atomic Energy Project.

fifteen rats (the controls) received vitamin D (7 units of calciferol [Drisdol] per gram of diet). After 27 days of the diets, x-ray examination showed normal bone-end development in the controls, while the experimentals showed the characteristic wide epiphyseal plates of rickets. Three days later, the animals were killed; the tibias were cut transversely at the midpoint of the shaft, and the proximal ends, including epiphysis, metaphysis, and some portion of shaft, were split in the longitudinal direction, washed with distilled water, and frozen for storage.

Except for a few specimens which were reserved for standard hematoxylin and eosin histological examination (which confirmed the x-ray findings), the bone sections were next demineralized at 37 C by suspending groups of four pieces each for seven days in three successive 250 ml. portions of the ethylenediamine-tetraacetic acid solution. Previously [4] this procedure removed all but small traces of mineral. By direct analysis, the average total content of eight pieces of bone was about 14μg. of calcium per section, or at least 99.7% demineralized.

The pieces of bone, in groups of eight, were placed in a sintered-glass funnel into which wash fluid was dripped: 300 ml. of the diluting solution (Solution C, above), followed by 500 ml. of distilled water, followed again by 300 ml. of buffered KCl (Solution C).

The bone sections, sliced by hand to a thickness of about 1 mm., were then individually placed in 10 ml. portions of mineralizing solution at 35 C without shaking. The solution was replaced every eight hours for a total of five times. After the final equilibration, the pH of the supernatant fluid was usually determined (no change in nonseeding solutions, small decrease to 7.2 in solutions from which sections took mineral out) and the bone sections were fixed for 14 to 18 hours, first in 95% then in absolute alcohol.[15] After fixation, the sections were stained for mineral content by the Pearse [16] modification of the von Kossa technique.[17]

This mineralizing procedure, slightly modified from that described earlier,[4] evolved from empirical experimentation. When large volumes of mineralizing solutions (150 ml. per bone piece) were employed, seeding in the bone resulted in a generalized precipitation throughout the flask. When smaller volumes (10 ml. per bone piece) were used, mineralization occurred in the bone section only. In such cases, however, the total mineral content of the solution was insufficient to produce more than a few crystals, hence the solutions were changed frequently.

Results

There were no observable differences in mineralization between the sections taken from normal rats and those taken from rachitic rats. In both cases, mineralization failed to occur at low products ($Ca \times P$, milligrams per cent, less than 40), while at higher products many crystalline aggregates were formed. These findings are very comparable quantitatively to results obtained earlier with normal bovine tissues.[4] The appearance of those sections which mineralized could scarcely be termed *normal*. Rather than a continuous even distribution of von Kossa stain, the mineral deposit appeared as innumerable globular masses closely adherent to decalcified trabeculae. That these spheroid deposits did not represent random precipitation is strongly indicated by the failure of the epiphyseal cartilage to mineralize *in all instances*. Apparently, once nucleation by the matrix had started, mineral deposition occurred as a self-sustaining process with growth on already formed crystallization centers in preference to the initiation of new centers. This artifactual distortion, while not unexpected at a cut *surface* of a section in vitro, could hardly occur to such an extent in vivo in the densely packed fibrous masses of trabeculae and osteones of living bone.

The results of the seeding experiments are summarized in the Table. Typical photomicrographs of tissue sections which did

Results of the Seeding Experiments

Bone Sections, Total No.*	Solution Composition			Degree of Mineralization ‡	
	Calcium, Mg. %	Phosphate, Mg. P %	Product, Ca×P †	Control	Exptl.
8	6.3	3.5	22	--	--
8	7.8	4.4	34	--	--
4	6.3	5.6	35	--	--
8	6.3	6.2	39	±	±
8	6.3	6.8	43	+	+
8	6.3	7.3	46	++	++
4	6.3	8	50	+++	+++
8	9.4	5.3	50	+++	+++

* Equally divided between controls and experimentals.

† This simple ion product may be converted to the thermodynamic ion product $a_{Ca^{++}} \cdot a_{HPO_4^{=}}$ by dividing by 18.6×10^7 (Strates et al).

‡ + indicates minimal calcification as determined by the presence of scattered spherical bodies of mineral adherent to decalcified trabeculae lying along both transverse borders of the epiphyseal plate. ++ and +++ designate greater degrees of calcification having the same distribution.

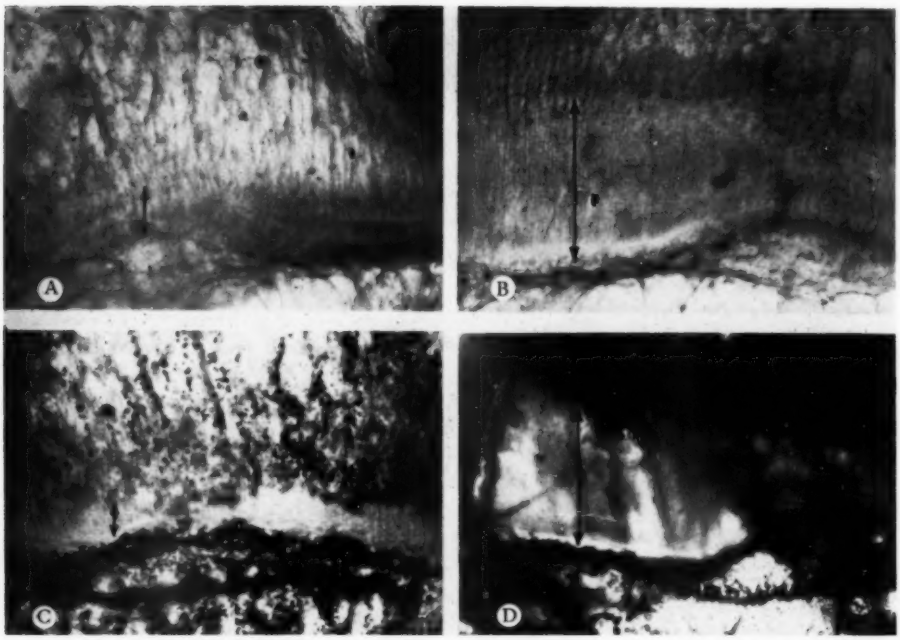


Fig. 1.—*A*, demineralized tibia from rat given control diet incubated at Ca×P product (milligrams per cent) of 35 (too low to induce mineralization). Note the narrow epiphyseal plate indicated by the arrow. Reduced about 55% from mag. × 50. *B*, rachitic tibia under same conditions as *A* but with Ca×P product of 22. Note the wide epiphysis. Reduced about 55% from mag. × 50. *C*, same as *A* but with Ca×P product of 49, showing deposition of mineral on mature trabeculae in the epiphysis (top) and diaphysis (bottom). The dense band of mineralization at the lower border of the nonmineralized epiphyseal plate probably represents the zone of provisional calcification. Von Kossa stain; reduced about 55% from mag. × 50. *D*, same as *B* but at Ca×P product of 49, showing mineralization which is distributed similarly to that of *C*. Reduced about 55% from mag. × 50.

and did not mineralize are given in Figures 1 and 2.

To provide a basis for a comparison of the seeding results with the observed failure of mineralization in the rachitic rats, analyses of serum for both calcium and inorganic phosphate were performed with the following results (averages of groups of six):

	Mg. % Ca	Mg. % P	Ca×P
Deficient	11.4	2.5	29
Normal	14.5	4.1	60

If it is assumed that the levels of serum protein were normal in both groups and, further, that the fraction of serum calcium which is ionized is the same in rats as in humans,[6] the *corrected* ionic products, corresponding to the test mineralizing solutions, would be 15 to 20 and 35 to 40 for the rachitic and control animals, respectively.

Comment

It was probably Howard[12] who first successfully remineralized sections of bone which had been demineralized with ethylenediamine-tetraacetic acid. There is some doubt, however, that his sections did not still contain residual crystals of bone mineral.[12] The present work, at face value, seems at variance with the experience of Sobel and co-workers,[13,14] who reported the failure of ethylenediamine-tetraacetic acid-treated bone to remineralize at products, Ca×P, of 60! In our hands, such products precipitate spontaneously[3] *in the absence of carbonate*. Whether these differences

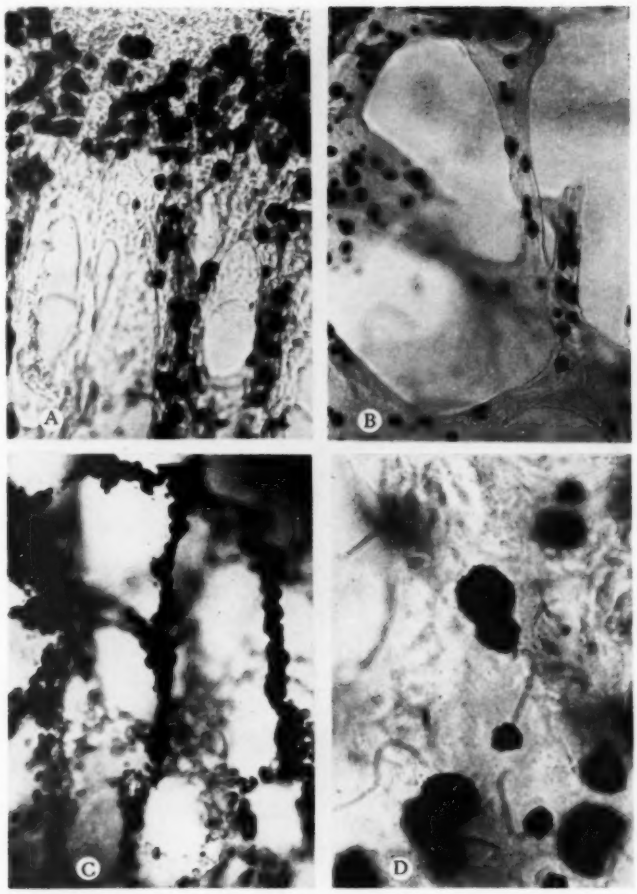


Fig. 2.—*A*, rachitic bone remineralized at Ca×P product of 50. Note the multiple spheroid deposits in close relation to the trabeculae, indicative of numerous distinct seeding sites; reduced 40% from mag. × 142. *B*, control bone, otherwise same as *A;* reduced 40% from mag. × 142. *C*, rachitic bone, showing area with dense deposition of mineral. Ca×P product of 49; reduced 40% from mag. × 142. *D*, rachitic bone at Ca×P product of 50, showing mineral aggregates with prickly borders; reduced 40% from mag. × 640.

are due solely to the presence or absence of carbonate, due to the criticality of solution volumes, or due to other as yet unsuspected differences in technique is not yet clear. At least two laboratories have successfully induced crystal formation from stable solutions of calcium and phosphate with highly purified collagen.[3,5]

Recently,[3] it was shown that only collagen, of several proteins, specifically possesses the ability to seed crystal formation. More recently,[5] it was found that only the native type of crystal form (640A axial repeat) of collagen is specifically able to induce the formation of hydroxy apatite crystals. It seems reasonable, therefore, to suppose it was the collagen present in the tissue sections which initiated mineral formation in these experiments. If this is true (it is far from proven), the present experiments provide a basis for two tentative conclusions: (*a*) A dietary deficiency of vitamin D does not seem to prevent the formation of collagen fibers which are nor-

mal with respect to crystal induction, and (*b*) the circulating levels of calcium and phosphate in the D-deficient animals are far too low to permit easy mineralization of collagen fibers in bone. In the control animals the levels of calcium and phosphate appear to be sufficiently high to permit mineralization of the collagen fibers *in the absence of local "booster mechanisms."* [7,11,14]

This is not to say that local cellular activity is not important in the calcification process and/or that vitamin D is not involved in such activity. It does seem, however, that the *matrix*, or more particularly the collagen, is not faulty in vitamin D deficiency, at least with respect to crystal induction.

Vitamin D by favoring calcium absorption [18] and a direct action on formed bone [6,19] raises the circulating levels of calcium and phosphate. It would appear from the present results that this is important, if not primarily responsible, for the stimulating action of the vitamin on the mineralization process, a possibility long recognized by researchers and clinicians.

The failure of epiphyseal cartilage to mineralize, as contrasted with trabecular osteoid and *probably* also the provisional zone of calcified cartilage (it is difficult to be certain), adds additional weight to the argument that the collagen molecule is important in inducing mineralization. It has been reported, on the basis of electron micrography, that the collagen in immature developing cartilage is poorly crystallized and poorly aggregated.[20]

Summary

Epiphyseal ends of rat tibiae reared with high-calcium low-phosphorus diets, with and without vitamin D supplements, were exhaustively demineralized by ethylenediamine-tetraacetic acid solutions at pH 7.4. The mineral-free collagenous tissue remaining was sectioned, and the slices were placed in inorganic solutions of varying calcium and phosphate contents. The slices, whether from rachitic or from normal animals, were equally efficient in inducing the formation of hydroxy apatite crystals from otherwise stable solutions. Though of limited interpretive significance, these results suggest that the failure of the mineralization processes in vitamin D deficiency is due to something other than an alteration of collagen structure and function.

Dr. R. Terry, Dr. L. Orbison, Dr. R. Moore, and Mr. B. S. Strates assisted in this study.

University of Rochester School of Medicine and Dentistry, Department of Pathology and Division of Pharmacology (45).

REFERENCES

1. Engfeldt, B., and Zetterström, R.: Biophysical Studies of Bone Tissue of Dogs with Experimental Rickets, A. M. A. Arch. Path. 59:321, 1955.

2. Neuman, W. F., and Neuman, M. W.: The Nature of the Mineral Phase of Bone, Chem. Rev. 53:1, 1953.

3. Strates, B. S.; Neuman, W. F., and Levinskas, G. J.: The Solubility of Bone Mineral: II. Precipitation of Near-Neutral Solutions of Calcium and Phosphate, J. Phys. Chem. 61:279, 1957.

4. Strates, B. S., and Neuman, W. F.: On the Mechanisms of Calcification, Proc. Soc. Exper. Biol. & Med., to be published.

5. Glimcher, M. J.; Hodge, A. J., and Schmitt, F. O.: Macromolecular Aggregation States in Relation to Mineralization: The Collagen-Hydroxyapatite System as Studied in Vitro, in Proceedings of the National Academy of Sciences, 1957, Vol. 43, p. 860.

6. Neuman, W. F., and Neuman, M. W.: Chemical Dynamics of Bone Mineral, Chicago, The University of Chicago Press, 1958.

7. Gutman, A. B., and Yu, T. F., in Metabolic Interrelations: Transactions of the First Conference, New York, Feb. 7-8, 1949, edited by E. C. Reifenstein Jr. and V. Johnson, New York, Josiah Macy Jr. Foundation, 1949, p. 11.

8. Gutman, A. B., and Yu, T. F., in Metabolic Interrelations: Transactions of the Second Conference, New York, Jan. 9-10, 1950, edited by E. C. Reifenstein Jr. and V. Johnson, New York, Josiah Macy Jr. Foundation, 1950, p. 167.

9. Nordin, B. E. C.: The Solubility of Powdered Bone, J. Biol. Chem. 227:551, 1957.

10. Neuman, W. F.; Firschein, H.; Chen, P. S.; Mulryan, B. J., and DiStefano, V. D.: On the Mechanism of Action of Parathormone, J. Am. Chem. Soc. 78:3863, 1956.

11. Burger, M.; Lavine, L. S.; Deane, B. C., and Sobel, A. E.: Calcification: XIX. Calcification of Transplanted Rachitic Bone, Proc. Soc. Exper. Biol. & Med. 96:147, 1957.

12. Howard, J. E.: Unpublished results.

13. Sobel, A. E., and Burger, M.: The Role of Chondroitin Sulfate in the Calcifying Mechanism, Clin. Chem. *2*:276, 1956.

14. Sobel, A. E.; Burger, M.; Deane, B. C.; Albaum, H. G., and Cost, K.: Calcification: XVIII. Lack of Correlation Between Calcification in Vitro and Glycolytic Enzymes, Proc. Soc. Exper. Biol. & Med. 96:32, 1957.

15. Yendt, E. R.; Connor, T. B., and Howard, J. E.: In Vitro Calcification of Rachitic Rat Cartilage in Normal and Pathological Human Sera with Some Observations on the Pathogenesis of Renal Rickets, Bull. Johns Hopkins Hosp. 96:1, 1955.

16. Pearse, A. G. E.: Histochemistry: Theoretical and Applied, London, J. & A. Churchill, Ltd., 1953, p. 485.

17. von Kossa, J.: VIII. Über die im Organismus künstlich erzeugbaren Verkalkungen, Beitr. path. Anat. *29*:163, 1901.

18. Nicolaysen, R., and Eeg-Larsen, N.: Biochemistry and Physiology of Vitamin D, in Vitamins and Hormones: Advances in Research and Applications, edited by R. S. Harris, G. F. Marrian, and K. V. Thimann, 1953, Vol. 11, p. 29.

19. Carlsson, A., and Lindquist, B.: Comparison of Intestinal and Skeletal Effects of Vitamin D in Relation to Dosage, Acta physiol. scandinav. 35:53, 1955.

20. Robinson, R. A., and Cameron, D. A.: Electron Microscopy of Cartilage and Bone Matrix at the Distal Epiphyseal Line of the Femur in the Newborn Infant, J. Biophys. & Biochem. Cytol. (Supp.) *2*:253, 1956.

Aneurysm of the Left Coronary Artery

Review of Literature and Report of Three Cases

AARON PLACHTA, M.D., and FRANCIS D. SPEER, M.D., New York

Aneurysms of the coronary arteries may exist as pathological curiosities of little clinical significance or remain unsuspected,[29,34,36-39,43-45] as in our cases, until discovered in the course of postmortem examination. Since Bougon's [4] first case report, 146 years ago, 53 adults and 14 infants and children, totaling 67 cases, were collected from the literature. Our contribution of 3 adult cases increases the number of adults to 56, making a total to date of 70 cases. Aneurysms of the coronary arteries have been the subject of study because of their rarity, their disputed causation, and their failure to produce typical symptoms.

The rarity of coronary artery aneurysms, exclusive of the false or dissecting forms, is stressed by Karsner.[21] Scott's [39] search of the existing files of 19,403 autopsied patients at the Mallory Institute of Pathology yielded only one example.

Packard and Wechsler [30] were unable to show any clinical findings on which to establish the diagnosis. They pointed out that aneurysms usually occur in the first part of the vessel concerned, or at the bifurcation of the vessels, and that the condition usually occurs singly and involves the left coronary more often than the right.

In view of the extremely high incidence of coronary sclerosis and the rarity of aneurysms, sclerosis can hardly be conceived as being the only factor involved in their production in the absence of hypertension. Scott's [39] review suggested the etiology of the coronary artery aneurysms as mycotic, embolic, atherosclerotic, traumatic, congenital, and syphilitic. Packard and Wechsler,[30] following their review of the literature, suggested the etiologic classification of these lesions into mycotic-embolic and arteriosclerotic. The inflammatory nature of the mycotic-embolic aneurysm was adequately described by Eppinger,[14] chiefly occurring in acute or subacute bacterial endocarditis of the aortic valve.

In the majority of cases described in the literature,[22,24,25,27] the coronary arteries, as in our cases, are cited as moderately or markedly tortuous and severely calcified, with the wall of the aneurysmal vessel either extremely thin,[1-3,11,12,25,27] exhibiting irregular hyaline deposits, or made rigid by calcified plaques.

Report of Cases

CASE 1.—The patient was a 73-year-old white known-hypertensive man. He was hospitalized on three occasions with blood pressures of 240/120, 210/130, and 230/110, respectively. He was readmitted to the hospital because of severe sudden deep abdominal pain radiating toward the lower extremities and weakness. Physical examination revealed a critically ill patient in shock, complaining of weakness and excruciating abdominal pain radiating toward the lower extremities. His blood pressure was 90/55; pulse, 140 and feeble; respirations, 24 and shallow; temperature, 96.2 F. He was treated symptomatically, but he lapsed into coma and died 18 hours following admission.

The gross pathological findings were as follows: atherosclerosis, generalized, severe; rupture of the abdominal aorta; retroperitoneal and subdiaphragmatic hemorrhage; atherosclerosis of coronary arteries, severe; aneurysm of coronary artery, anterior, descending, left; hypertrophy, concentric, of myocardium; pneumonitis; pulmonary edema, atelectasis, and emphysema; fatty metamorphosis

Submitted for publication March 3, 1958.

From the Department of Pathology and Clinical Pathology, Flower and Fifth Avenue Hospitals, New York Medical College Metropolitan Medical Center.

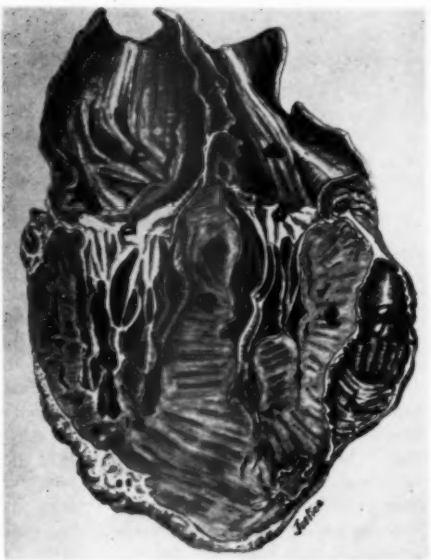


Fig. 1.—(*a*) Left dilated atrium. (*b*) Mitral valve. (*c*) Right severely dilated atrium. (*d*) Tricuspid valve. (*e*) Interventricular septum and hypertrophied right and left ventricular myocardium. (*f*) Broad, widened, calcified, proximal segment of the anterior descending left coronary artery, resting on the (*g*) ballooned oval-shaped aneurysm with unobstructed lumen. (*h*) Partially obstructed thrombotic apex of aneurysm, with the (*i*) continuous severely sclerotic distal segment of the coronary artery.

of liver; arteriolar nephrosclerosis; subcortical cysts, bilateral; nodular hyperplasia of prostate gland; arteriosclerosis of cerebral arteries.

The heart weighed 680 gm. (Fig. 1.) The pericardial cavity contained no excess fluid, and surfaces were glistening. The heart measured 15.6 cm. across the base and 13.5 cm. from the base to the rounded apex. The rounded apex was made up of both right and left ventricles. The right atrium was moderately dilated. The right ventricular wall was 0.9 cm. in thickness. The foramen ovale was obliterated. The left atrium was dilated. The left ventricular wall was dark brown and 2.3 cm. in thickness, showing also subendocardial fibrosis. The bulky papillary muscles in both right and left myocardium attested to the severe concentric hypertrophy characterized by the globular appearance. All valves were essentially normal except the aortic, which showed moderate calcification in the absence of stenosis. The left coronary ostium was dilated and measured 2.1 cm. in diameter. The first part of the anterior descending coronary artery was characterized by a broad, widened, calcified, patent, 1.9 cm. luminal diameter, tortuous proximal segment, resting on an intermediate ballooned oval-shaped 5.8×2.9 cm. aneurysm. The wall of the base of the aneurysm measured 0.3 cm. in thickness and was fibrocalcific. The lumen was unobstructed, permitting a free flow of blood. The maximum luminal diameter (2.9 cm.) of the aneurysm was at the midportion. The wall at this point was 0.1 cm. in thickness. The partially obstructed thrombotic apex of the aneurysm was continuous with the severely sclerotic, narrow, 0.3 cm. luminal diameter, distal segment of the coronary artery. The right coronary artery was calcified and ulcerated. The lumen was patent.

The thoracic and abdominal aorta was severely calcified and ulcerated. The lumbar segment of the abdominal aorta was the seat of an oval calcified thrombosed aneurysm that measured 12.8×7.4 cm. There was a pinhead-sized rupture of the left posterior medial aspect in the area of the fifth vertebra, with massive retroperitoneal and subdiaphragmatic hemorrhage involving both sides.

Radiopaque studies of the heart revealed marked interarterial and venous anastomosis. These intercoronary arterial and venous plexuses served to illustrate the extent of blood flow to the hypertrophied myocardium. The dilated proximal portion of the anterior left descending coronary artery freely emptied into the broad oval-shaped atherosclerotic aneurysm of the anterior descending

Fig. 2.—(*a*) Marked interarterial and venous anastomosis. (*b*) Dilated proximal descending left coronary artery, freely emptying into the (*c*) broad ballooned oval-shaped atherosclerotic aneurysm leaving the (*d*) apex of the partially thrombosed aneurysm of the coronary artery.

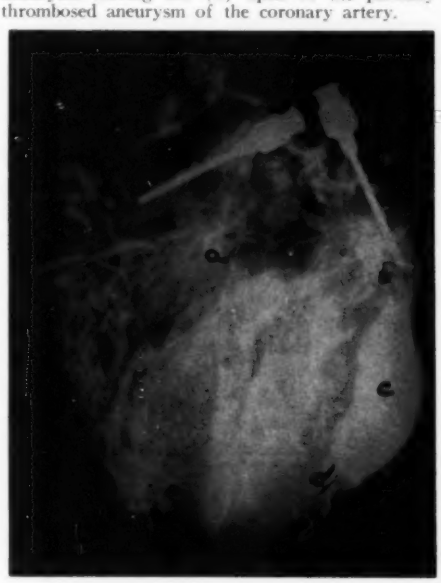


Fig. 3.—(*a*) Dilated ostium of the left circumflex coronary artery. (*b*) Severe myocardial hypertrophy. (*c*) Calcification of coronary arteries and thoracic aorta.

coronary artery. The distal coronary artery segment attached to the apex of the partially thrombosed aneurysm was narrow, tortuous, and incompletely occluded (Fig. 2).

CASE 2.—The patient was a 76-year-old white hypertensive man (blood pressure 240/130) who entered the hospital because of chills, fever, and rusty productive cough. In spite of chemotherapy and antibiotics, he died on the fourth hospital day.

Gross pathologic findings were as follows: atherosclerosis, generalized, severe; calcification and ulceration of the thoracic and abdominal aorta; atherosclerosis of the coronary arteries; aneurysms of left circumflex and right coronary arteries; hypertrophy of left and right ventricular myocardium, with myocardial fibrosis; bilateral lobar pneumonitis; bronchiectasis, left lower lobe; tuberculosis, calcified, apical, right, healed; hydrothorax, right; splenomegaly, septic; hepatomegaly with fatty metamorphosis; arteriolar nephrosclerosis, bilateral; adenomata of adrenal glands; healed gastric ulcer; nodular hyperplasia of prostate gland.

The weight of the heart was 450 gm. The ostium of the left circumflex coronary artery was dilated, and the diameter of its proximal segment measured 1.7 cm., whereas its distal segment measured 0.2 cm. in diameter. Ballooned aneurysms of the right anterior and left circumflex coronary arteries measured 2.3×1.2 cm. and 3.7×1.8 cm., respectively. The pattern of both aneurysms was that of hyaline, calcified, thick walls and unobstructed lumina. Detailed gross description of the heart is omitted (Figs. 3 and 4).

CASE 3.—The patient, an 85-year-old white male known hypertensive, entered the hospital in coma and died three days later. The gross pathologic findings were as follows: generalized atherosclerosis; calcification and ulceration of the thoracic and abdominal aorta; atherosclerosis of coronary arteries; aneurysm of left circumflex coronary artery; serofibrinous pericarditis; hypertrophy, concentric, of myocardium, and myocardial fibrosis; pulmonary edema and bilateral bullous emphysema; focal pneumonitis; splenomegaly; arteriolar nephrosclerosis; subcortical cysts, bilateral; nodular hyperplasia of prostate gland; hydrocele; diverticulosis of sigmoid and transverse colon; adenomatous pedunculated polyps of sigmoid colon; saccular aneurysm of right middle cerebral artery; hemorrhage into lateral ventricles; encephalomalacia, hemorrhagic, right temporal-parietal; arteriosclerosis of cerebral arteries.

Except for the weight of the heart, 810 gm.; site of dilated ostium and proximal segment, 1.5 cm. luminal diameter, and the corresponding narrow, 0.3 cm. luminal diameter of the distal sclerotic segment of the left circumflex coronary artery, the intermediate calcified ballooned 2.9×1.5 cm. aneurysm had a pattern similar to that observed in the previously described coronary artery aneurysms (Fig. 5).

Fig. 4.—(*a*) Ballooned, hyaline calcified partially thrombosed aneurysm of the left circumflex coronary artery. (*b*) Unobstructed lumen.

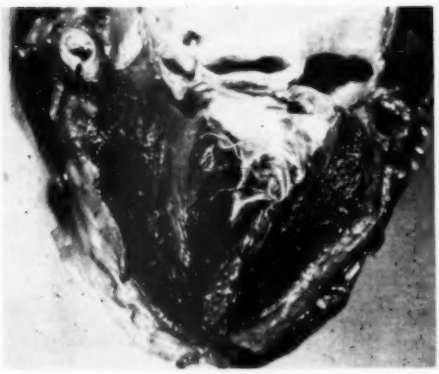


Fig. 5.—(*a*) Severely dilated left ostium. (*b*) Ballooned, severely calcified partially thrombosed aneurysm of circumflex left coronary artery. (*c*) Hypertrophied myocardium.

Gross Findings

The study of the hearts and coronary trees of our three cases is given below in the form of a composite analysis of the morphologic findings.

The coronary arteries aside from those areas with aneurysmal dilatation were characterized by narrowed and tortuous vessels showing moderate to advanced atherosclerosis. In many instances the intima showed calcification with or without ulceration. The lumina of many segments of these vessels were partially or completely occluded by organized old or fresh thrombi. The hypertrophied myocardium revealed related focal myocardial fibrosis of old and recent myocardial infarctions.

In contrast to the lesions observed in the arteries not involved by aneurysm, the coronary arteries showing aneurysms revealed moderate to severe dilatation of the respective ostia of the involved vessels. Luminal dilatation of the proximal segment of the artery preceded the ballooned portion of the aneurysm. These segments revealed inconspicuous to moderate atherosclerotic intimal involvement. The aneurysms were roughly fusiform or saccular and showed atherosclerosis of their walls with, in most instances, an organized thrombus partially occluding their distal recesses. The distal segment of the involved coronary artery was invariably narrowed by atherosclerotic and thrombotic changes. The anatomic outline, therefore, suggested a patent dilated proximal artery segment, an intermediate ballooned oval-shaped or rounded body of the aneurysm, and a narrowed atherosclerotic distal segment of the artery. This configuration is well demonstrated by the radiopaque studies.

Microscopic Findings

Multiple sections from the right and left coronary arteries of the distal and proximal segments (Fig. 6) and the aneurysmal areas as well were prepared with Van Gieson, Weigert, Mallory aniline blue, Verhoeff, and hematoxylin and eosin stains to determine the extent of atherosclerotic involvement and the presence of other changes that might suggest factors in the pathogenesis of the focal dilatation.

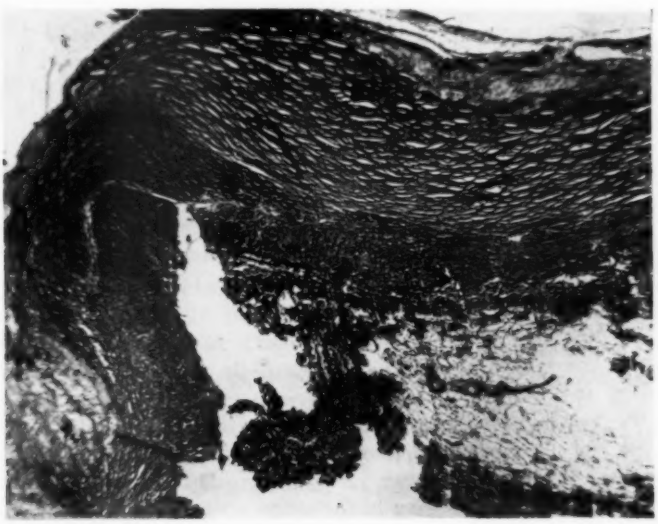


Fig. 6.—Eccentric and annular atheromatous plaques, cholesterol crystals, and lipophages.

In all three hearts sections of the nonaneurysmal tortuous coronary arteries showed eccentric and annular atheromatous plaques. These lesions were characterized by cholesterol crystals, lipophages, amorphous material, hemosiderin, and granules of calcium phosphate. In instances the atheromatous deposits were covered by zones of hyalinized connective tissue which outlined the narrowed lumen. In some sections fresh or organized thrombi filled small or large lumina of sclerotic arteries.

Multiple sections of the walls of the aneurysms (best illustrated by Mallory's aniline blue collagen stain, Zenker-fixed tissue) revealed partially hyalinized media and proliferative calcified intima. As the study of sections approached the midportion or site of the maximum luminal dilatation of the aneurysm, fragmentation of cholesterol clefts and absence of intimal elastic lamina, with partial or complete hyalinization of atrophic media and adventitial fibrosis, were observed. Destruction of the inner elastic membrane and the necrotic atheromatous material with conspicuous calcification were best observed with the combined Van Gieson and Weigert stain or Van Gieson stain for collagen fibers.

Verhoeff's elastic stain best illustrated the findings of the coronary arteries distal to the dilated tortuous aneurysmal portions, which showed marked intimal atherosclerosis, reduplication, and focal breaking of the internal elastic lamella; hyalinized broad media, and necrotized poorly defined external elastic lamella. Verhoeff's stain applied to the coronary arteries proximal to the aneurysm showed uniform intimal thickening. The internal elastic lamella was not reduplicated but exhibited marked fragmentation. The diminished elastic tissue and muscular atrophy of the media were characterized by the nearly straight fibers. The external elastic lamella showed thinning and fragmentation. The muscle cells in some places were hypertrophic and grouped in bundles with intervening bands of collagen.

Comment

Attempts to clarify the pathogenesis of vascular aneurysm are unsettled. Forbus [16] suggests that they occur on the basis of congenital defects, pointing to the inadequate development of the muscular layer, chiefly at the point of bifurcation. This he feels is true in cerebral vessels with the so-called congenital or "berry" aneurysm which has become widely accepted as pointed out by Green [18] and Padget.[31] Forbus implied that a similar developmental defect may be operative in the coronary arteries. This contention is supported by Harris.[19] Glynn,[17] however, emphasizes that the development of an aneurysm in a given vessel follows a disturbed elastica, regardless of the etiology. He feels that the frequency of aneurysm located at points of bifurcation is due to fusion of the muscularis in embryonic life which is apt to be incomplete and it is here that the elastic lamina is the weakest.

Packard and Wechsler place coronary artery aneurysm into two etiological groups: (1) mycotic-embolic, associated with bacterial endocarditis involving the aortic valve, and (2) atherosclerotic, related to coronary sclerosis, associated with long-continued hypertension.

Scott's classification of aneurysms of the coronary artery, following his review of the literature, results in the coding of 15 case reports as congenital. These lesions were either localized or diffuse. The localized were either saccular or fusiform, and the diffuse were dilated and tortuous.

Mozen's [28] recent addition bears the title "Congenital Cirsoid Aneurysm of a Coronary Artery with Associated Atrio-Atrial Fistula, Treated by Operation." The 10-year-old boy was operated on, resulting in the obliteration of the fistula and the disappearance of what was considered the aneurysm of the coronary artery. According to the author's description of the posi-

tion and character of the involved coronary artery, the anomaly, we believe, was basically that of the fistula precipitating the uniform dilatation of the vessels, as illustrated in the report. We are inclined to believe that a uniform vascular ectasia secondary to an anomalous atrioatrial fistula should not be regarded as an example of coronary artery aneurysm.

Valdivia and associates [46] recorded a large coronary artery aneurysm arising at the terminal end of the right coronary artery communicating with the right atrium. The aneurysm was thrombosed, and part of the wall, calcified. The authors considered congenital origin as most logical.

Crocker and associates [9] list 67 case reports collected from the literature. Most of the cases occurred in adults, with the exception of the mycotic embolic variety [6,20,23] and those associated with rheumatic carditis [23,35] or with polyarteritis nodosa,[33,40] which occurred in both adults and children. The total included their report of three cases of coronary artery aneurysm in infants. One case was associated with malformation of the right coronary artery and the right side of the heart. This case they considered to be developmental, while the mechanism of aneurysm formation in the two other cases was classified as undetermined.

There is no doubt that abnormal development plays a part in the formation of localized aneurysms of the coronary arteries in infancy, and the assumption of such etiology is warranted in cases in which there are other congenital cardiovascular anomalies.[9,26,42] In such instances the patient rarely survives over a period of 12 months.

In the coronary arteries of older age groups [5,7,8,11-13,15] there is little evidence that a developmental defect of the media is responsible for the formation of aneurysms. The destruction of the elastic fibers and muscular atrophy at or proximal to the site of atheromas seems to be an important factor which could lead to aneurysm formation.

The introduction of sulfonamides, antibiotics, and, particularly, penicillin has changed the course of infectious disease. In the past decade our autopsy files have shown a progressive decline of cases having subacute or acute valvular lesions. This altered course of endocarditis should tend in the future to reduced further the already rare mycotic aneurysm of the coronary arteries.

A similar reduction of coronary aneurysm would apply to the rare complication of the coronary arteries in the inflammatory allergic diseases (collagen diseases), such as periarteritis nodosa, showing a favorable therapeutic response to the recent introduction of corticotropin (ACTH) and cortisone.

To include syphilitic arteritis [10] as a cause of aneurysm is to overlook the generally accepted belief that the coronary arteries are only rarely, if ever, affected by syphilis distal to their intra-aortic segments. One of Snyder and Hunter's [41] cases, despite failure to demonstrate the organism by spirochete stains and advanced atherosclerosis of pulmonary arteries with aortic involvement far greater than observed in syphilis, is included in the group of syphilitic etiology.

The authors, following a review of case reports in addition to their own three cases, intend to imply that atherosclerosis and hypertension should be considered as the major pathogenetic factors in the formation of coronary aneurysms.

Our studies lend support to the view of atherosclerosis as a basis of coronary aneurysm in older patients. No developmental medial muscular defect was observed in our cases. Elastic tissue stains revealed focal fraying of the elastica of the saccular aneurysm and complete disappearance of elastic fibers within the aneurysmal sac. The uninvolved portions of the vessels showed a preserved although altered elastica. There is little doubt that the destruc-

tion of the media is due to slowly expanding intimal atheroma.

On the basis of our study, not overlooking the infrequent possibility that developmental factors may play a part in coronary aneurysmal lesions, we are suggesting three major groups: 1. Arteriosclerotic, as related to focal coronary sclerosis, associated with long-standing hypertension; degeneration and atrophy of media associated with the loss of intima. In 50% of these patients the aneurysms terminate in rupture and tamponade; 45% have a prolonged asymptomatic course ending in myocardial exhaustion, insufficiency, and failure, and 4% terminate in thrombosis and infarction. In these cases the left coronary artery is more frequently involved and the aneurysm is usually single. 2. Embolic-mycotic, in which no predilection as to age and sex exists and which are related to an inflammatory lesion produced by a variety of organisms chiefly occurring in acute and subacute bacterial endocarditis of the aortic valves. 3. Congenital malformation. The congenital group is usually found in association with other cardiovascular or visceral malformations.

Summary

To date 53 adult cases of aneurysms of the coronary artery have been reported. Three additional examples of aneurysm of the left coronary artery, all occurring in men in the seventh and eighth decades and all in advanced arteriosclerotic hypertensive patients, are recorded.

The literature is reviewed, and the rarity of the lesion is reemphasized.

On the basis of study, a pathogenetic classification of aneurysm of the coronary arteries into three major groups is proposed, namely: (1) arteriosclerotic, related to coronary sclerosis associated with long-standing hypertension; (2) embolic-mycotic, related to the inflammatory lesions associated with bacterial endocarditis, usually of the aortic valve, and (3) congenital malformation, an obscure lesion, usually terminating in myocardial infarction or tamponade and rare survival over a period of 12 months.

New York Medical College, Flower and Fifth Avenue Hospitals, Laboratory of Pathology (9).

REFERENCES

1. Ashton, N., and Munro, M.: Coronary Artery Aneurysm with Occlusion Due to a Calcified Thrombus, Brit. Heart J. 10:165-166, 1948.

2. Belsky, H.; Sharnoff, J. G., and Gilbert, S.: Aneurysm of the Coronary Artery, New York J. Med. 55:3151-3152, 1955.

3. Blankenhorn, M. A., and Gall, E. A.: Clinicopathologic Conference, Am. J. Clin. Path. 25:543-553, 1955.

4. Bougon, A.: Arterial Aneurysm of the Heart, Biblioth. med. 37:183-185, 1812.

5. Bowden, K. M.: Coronary Aneurysm: Report of Case, M. J. Australia 1:209-211, 1949.

6. Clarke, J. J.: Embolic Aneurysm of the Heart, Buttock and Axilla, Tr. Path. Soc. London 47:24-25, 1896.

7. Colbeck, J. C., and Shaw, J. M.: Coronary Aneurysm with Arteriovenous Fistula, Am. Heart J. 48:270-274, 1954.

8. Cox, R. L., and Chester, D.: Aneurysm of the Coronary Arteries, Am. J. M. Sc. 180:37-42, 1930.

9. Crocker, D. W.; Sobin, S., and Thomas, W. C.: Aneurysm of the Coronary Arteries: Report of 3 Cases in Infants and Review of the Literature, Am. J. Path. 33:819-837, 1957.

10. Denham, S. W.: Syphilitic Aneurysm of the Left Coronary Artery, A. M. A. Arch. Path. 51: 661-665, 1951.

11. Dock, W.: The Predilection of Atherosclerosis for the Coronary Arteries, J. A. M. A. 131:875-878, 1946.

12. Dordi, F.: Descrizione di un raro caso di aneurisma dell'arteria coronarica sinistra, Pathologica 42:239-244, 1950.

13. Emminger, E.: Arterio-venöses Aneurysma der rechten Herzkranzschlagader, Klin. med. 2: 652-656, 1947.

14. Eppinger, H.: Pathogenesis (Histogenesis und Aetiologie) der Aneurysmen einschliesslich des Aneurysma equi verminosum, Arch. klin. Chir. 35:1-563, 1887.

15. Fangman, R. J., and Hellwig, C. A.: Histology of Coronary Arteries in Newborn Infants, Am. J. Path. 23:901-902, 1947.

16. Forbus, W. D.: On the Origin of Miliary Aneurysm of the Superficial Cerebral Arteries, Bull. Johns Hopkins Hosp. 47:239-284, 1930.

17. Glynn, L. E.: Medial Defects of the Circle of Willis and Their Relation to Aneurysm Formation, J. Path. & Bact. 51:213-222, 1940.

18. Green, F. H. K.: Congenital Aneurysm of the Cerebral Arteries, Quart. J. Med. 21:219-425, 1928.

19. Harris, P. N.: Aneurysmal Dilatation of the Cardiac Coronary Arteries: Review of the Literature and Report of a Case, Am. J. Path. 13:89-98, 1937.

20. Johnson, T. D., and Hurst, J. W.: Bacterial Endocarditis Due to Penicillin-Resistant Staphylococcus: Report of Case Successfully Treated with Erythromycin, Oxytetracycline and Streptomycin, New England J. Med. 251:219-221, 1954.

21. Karsner, H. T.: Coronary Arteriosclerosis, New York, The Macmillan Company, 1933, pp. 431-451.

22. Kempton, J. J.: Calcified Aneurysms of the Coronary Arteries, Proc. Roy. Soc. Med. 44:733-734, 1951.

23. Klinge, F., and Vaubel, E.: Das Gewebsbild des fieberhaften Rheumatismus: Die Gefässe beim Rheumatismus, insbesondere die "Aortitis rheumatica" (mit Betrachtung zur Ätiologie des fieberhaften Rheumatismus vom pathologisch-anatomischen Standpunkt), Arch. path. Anat. 281: 701-747, 1931.

24. Loring, W. E.: Multiple Arteriosclerotic Aneurysms of Coronary Arteries, A. M. A. Arch. Path. 59:449-452, 1955.

25. Malet, E.: Aneurysm of Coronary Artery: Rupture; Necropsy; Remarks, Lancet 2:67, 1887.

26. Minder, W. H.: Aneurysma congenitum Arteriae coronariae, Cardiologia 22:35-44, 1953.

27. Mitchell, N.: Arteriosclerotic Aneurysm of the Cardiac Coronary Arteries: Report of Case, Am. Heart J. 33:112-120, 1947.

28. Mozen, H. E.: Congenital Cirsoid Aneurysm of a Coronary Artery with Associated Atrio-Atrial Fistula, Treated by Operation: A Case Report, Ann. Surg. 144:215-217, 1956.

29. O'Neill, J. B., and Laipply, T. C.: Aneurysms of Coronary Arteries: Report of 2 Cases, Quart. Bull. Northwestern Univ. M. School 23:77-82, 1949.

30. Packard, M., and Wechsler, G. F.: Aneurysm of the Coronary Arteries, Arch. Int. Med. 43:1-14, 1929.

31. Padget, D. H.: The Development of the Cranial Arteries in the Human Embryo, Contrib. Embryol. 32:205-262, 1948.

32. Paul, O.; Sweet, R. H., and White, P. D.: Coronary Arteriovenous Fistula: Case Report, Am. Heart J. 37:441-445, 1949.

33. Pickward, C. M.; Owen, J. G., and Dammin, G. J.: Aneurysm of the Coronary Arteries Due to Polyarteritis Nodosa Occurring in an Infant, J. Lab. & Clin. Med. 32:1513-1514, 1947.

34. Prentice, A. I. D., and Penfold, J. B.: Aneurysm of the Coronary Arteries, Brit. Heart J. 14:87-90, 1952.

35. Rae, M. V.: Coronary Aneurysm with Thrombosis in Rheumatic Carditis: Unusual Occurrence Accompanied by Hyperleukocytosis in a Child, Arch. Path. 24:369-376, 1937.

36. Rigdon, R. H., and Vandergriff, H.: Aneurysm of the Coronary Arteries: Review of the Literature and Report of a Case, Am. J. Surg. 61:407-413, 1943.

37. Rukstinat, G. J.: Multiple Aneurysms of the Right Coronary Artery: Death from a Ruptured Aneurysm of the Abdominal Aorta, J. A. M. A. 149:1129-1131, 1952.

38. Sarkisian, S. S.: Aneurysm of the Coronary Artery: Report of a Case, U. S. Armed Forces M. J. 1:1281-1283, 1950.

39. Scott, D. H.: Aneurysm of the Coronary Arteries, Am. Heart J. 36:403-421, 1948.

40. Sinclair, W., Jr., and Nitsch, E.: Polyarteritis Nodosa of the Coronary Arteries: Report of Case in Infant with Rupture of Aneurysm and Intrapericardial Hemorrhage, Am. Heart J. 38:898-904, 1949.

41. Snyder, G. H., and Hunter, W. C.: Syphilitic Aneurysm of the Left Coronary Artery with Recurrent Aneurysm of Sinus of Valsalva, Am. J. Path. 10:757-772, 1934.

42. Taussig, H. B.: Congenital Malformations of the Heart, Cambridge, Mass., Harvard University Press, 1947, p. 618.

43. Tellem, M., and Rubenstone, A. I.: Aneurysm of the Left Coronary Artery, Am. J. Med. 19:652-654, 1955.

44. Trever, R. S.: Aneurysm of the Descending Branch of the Right Coronary Artery and Non-Valvular Infective Endocarditis, Proc. Roy. Soc. Med. 5:20-26, 1911.

45. Trinidad, S.; Grayzel, D. M.; Rapoport, P., and Lee, N.: Aneurysm of the Coronary Artery, Ann. Int. Med. 39:1117-1124, 1953.

46. Valdivia, E.; Rowe, G. G., and Angevine, D. M.: Large Congenital Aneurysm of the Right Coronary Artery, A. M. A. Arch. Path. 63:168-171, 1957.

Fatty Acid Supplements to the Cholesterol-Fed Rabbit

Effect on Serum Lipids and Atherosclerosis

CAPT. WALTER M. BORTZ (MC), U. S. Army (Res.); NILS PAUL LARSEN, M.D., and W. HAROLD CIVIN, M.D., Honolulu

Dietary fat has been accorded a central role in the pathogenesis of atherosclerosis. It has not been until recently that the qualitative, as well as the quantitative, aspects of fat ingestion have been appreciated. The final practical application of this appreciation to the processes of human atherosclerosis is not clear as yet. However, in experimental animals the deposition of arterial plaques has been shown to be markedly affected by the fat type as well as its abundance. In addition, in human and animal studies the serum cholesterol levels are seen to be influenced by alteration in the types of dietary fat provided.

Strict vegetarians have been noted to exhibit low serum-cholesterol values.[1] Schonheimer is said to have advised his patients with elevated cholesterol levels to adopt a vegetable-fat program as early as 1933.[2] Others came to this realization as well, and so a few years ago mention of the relative value of vegetable fat in contrast to animal fat was commonplace. Initially, vegetable fat was considered to be merely not as atherogenic as the animal counterpart, but the concept soon evolved that vegetable fats were not only less harmful but that they exerted, in some way, a protective influence against deposit in the arteries. The possibilities that this was due to the presence of cholesterol-inhibiting sterols or to phospholipids were discarded in favor of that of the unsaturated fatty acids.[3] Fats of vegetable origin are known to be characterized by a high proportion of unsaturated fatty acids. Nearly all studies on this subject have been directed toward the correlation of the iodine number of a fed fat with its effect on atheroma deposit and/or serum cholesterol levels.[4]

Few studies have utilized the fatty acids themselves as a provocative test. To this end the following experiment was conducted.

Submitted for publication Feb. 14, 1958.

The USARPAC Medical Laboratory provided technical assistance.

This work was supported by a grant from the Hawaii Heart Association.

Experiment

Twenty-four Albino rabbits of the Australian strain were used. Their initial weights varied from 2.20 kg. to 3.09 kg. Both sexes were represented, but the groups to receive a fatty acid supplement were all males; the other groups had male and female members. For six weeks the animals were maintained with Purina Rabbit Checkers ad libitum. The composition of this food includes a 2% fat minimum. No further breakdown on this was available. After six weeks of this feeding, 20 animals were started on a cholesterol supplement. This was calculated individually at 0.5 gm. of cholesterol per kilogram of body weight per day. This is a high dosage. The cholesterol was dissolved in ether; it was coated on the rabbit meal, and the ether was allowed to evaporate. The feed was prepared in weekly batches, and that which remained from the previous week was incorporated into that of the following week. Inasmuch as the animals became anorectic toward the end of the experiment, this was an important consideration.

At the end of five weeks of high-cholesterol feeding two animals were killed by exsanguination and the aortas and livers were examined. The animals were then grouped further. Four received 1 gm. of stearic acid supplement each per week. Four received 1 ml. of oleic acid per week, and four received 1 ml. of linolenic acid per week. (Stearic acid is solid, whereas oleic and linolenic are liquid. All have 18 carbon atoms in their chains.) Four continued the normal chow diet, and six received the high-cholesterol diet alone. After 18 days of this program another of the rabbits given the high-cholesterol diet was killed

TABLE 1.—*Mean Weights in Kilograms*

	Day	Normal	High Cholesterol Alone	Cholesterol & Stearic Acid	Cholesterol & Oleic Acid	Cholesterol & Linolenic Acid
Start	1	2.66	2.53	2.66	2.78	2.41
Normal diet	41	3.21	3.41	3.49	3.18	2.84
Cholesterol priming	75	3.50	3.87	4.17	3.42	3.09
Start of fatty acid	94	3.56	3.87	4.17	3.48	3.29
Day of experiment	108	3.63	3.83	4.12	3.46	3.27
End of experiment	138	3.80	3.72	4.05	3.41	3.24

and the liver and aorta, inspected. It had been contemplated to continue the fatty acid regimen for six weeks, but the animals became anorectic. Instead of the planned 42 days the rabbits required 62 days to consume six weeks of the diet outlined above. Accordingly, they each received 6 gm. or 6 ml. over a period of 62 days. At that time, all the remaining animals were killed and their liver and aortas, inspected.

During the course of the experiment the animals were weighed every two weeks, and the cholesterol dosage was adjusted accordingly.

Likewise, at pertinent times blood was withdrawn by direct cardiac puncture. This was analyzed for total cholesterol by the method of B. Zak. Lipid phosphorus (Youngsburg and Youngsburg) was determined, as were the lipids (de la Huerga).

At autopsy the aorta was divided at the level of the diaphragm and opened (longitudinally) opposite the points of emergence of the intercostals. The atheromatous deposits were graded separately in the arch and in the descending portion. A scale of 0 to 4 was utilized: 0 represents no lesions; 1 represents discrete, flat, widely scattered deposits; 2 represents an intermediate between 1 and 3; 3 represents lesions of more general distribution, with projection intraluminally and some confluence; 4 represents heavy deposit with marked heaping of lesions with complete or nearly complete covering of the intimal surface, with considerable intraluminal projection. No hemorrhage or ulceration was noted in any lesion. The grading was done by one of us (W. H. C.) without the knowledge of the group represented by each.

Results

(*a*) *Weight Gain.*—Table 1 represents the average absolute weights of the rabbits in the different stages of the experiment. It is noted that the changes are nearly parallel in all groups. All groups fed the cholesterol supplement, with or without the fatty acid addition, showed a weight gain until the end of the cholesterol priming, or two weeks of the fatty acid addition. Thereafter, each group exhibited a slight weight loss toward the end of the experiment. The normal group gained steadily throughout.

(*b*) *Serum Levels.*—Table 2 represents the total serum cholesterol values at the times shown. It is most noteworthy to observe that at the end of the cholesterol-priming phase all groups receiving cholesterol orally showed markedly elevated serum levels. At the end of the experiment, the groups receiving the fatty acids with unsaturated chains appear to have maintained this increase as well as the groups given cholesterol alone and cholesterol plus stearic acid. The higher cholesterol levels in the rabbits receiving the unsaturated fats appear significant, but the small number of animals forbids further interpretation. The serum lipid phosphorus and total lipid levels showed no apparent difference in any of the high-cholesterol-fed groups.

(*c*) *Aortas and Livers.*—The grading of the individual aortas is presented in Table 3. One of the stearic acid-fed rabbits died

TABLE 2.—*Total Serum Cholesterol in Milligrams Per Cent*

	Day	Normal	High Cholesterol Alone	Cholesterol & Stearic Acid	Cholesterol & Oleic Acid	Cholesterol & Linolenic Acid
Initial	1	131	137	126	122	116
Observation period	41	127	120	132	95	87
Cholesterol priming	75	166	1,423	1,697	1,643	1,750
Fatty acid added	94	151	1,755	2,105	1,933	2,085
Day of experiment	108	132	1,700	1,647	1,795	1,845
End of experiment	138	205	1,632	1,553	2,080	1,950

TABLE 3.—*Atherosclerosis in Aorta—Arch and Descending—in Individual Animals*

Normal	High Cholesterol Alone	Cholesterol & Stearic Acid	Cholesterol & Oleic Acid	Cholesterol & Linolenic Acid
0-0	3-3	4-1	3-1	4-1
0-0	4-3	4-3	3-1	3-1
0-0	4-3	4-3	2-2	3-1
0-0	4-4	3-3 (died Day 108)	4-1	4-2
	4-4			
	0-0 } Killed after 4 wk.			
	0-0 } of cholesterol supplement			
	3-1 Killed after 8 wk.			
	of cholesterol supplement			

during the cardiac puncture performed after 34 days of fatty acid feeding. Its aorta was similarly graded and is here included. It is noted that in all cases the severity of the lesions in the arch was more than in the thoracic portion. This confirms the observation of many others. It is also to be noted that in every case with the exception of one stearic acid-fed rabbit those receiving an unsaturated acid additive showed markedly less atheroma deposit. This observation is valid in the most critical sense, the grading being reevaluated in the light of the experimental conditions applied and the previous rating, confirmed. The pictures are of the individual aortas after a formalin fixative.

Fig. 1.—Normal aorta.

The explanation of the findings in the stearic acid exception is not clear. The rabbit's weight paralleled that of the rest of the group, as did its serum lipid values until the terminal bleeding values. At this time, all the lipid fractions measured were lower than those of any other animal receiving the cholesterol. Why this is so is not evident.

The livers of all the animals fed the cholesterol supplement appeared similar, showing mottled dull red and yellowish color, with two exceptions. They were in the linolenic acid group. Their livers appeared a homogeneous salmon color. Aside from this they could not be distinguished from the other members of their group.

Comment

Early studies claimed that those patients maintained with a low-fat diet had increase in serum cholesterol and β-lipoprotein levels when vegetable fats were incorporated in the diet.

This has been disputed. Ahrens' and Kinsell's groups have noted falls in serum cholesterol levels of patients fed vegetable oils, even in high quantities.[1,3,5] Extending this observation, Ahrens et al.,[6] Bronte-Stewart et al.,[7] and Beveridge et al.[8] have fed various vegetable oils with different iodine numbers. They have been able to demonstrate a correlation between the amount of unsaturation of a fat and its ability to lower serum cholesterol.

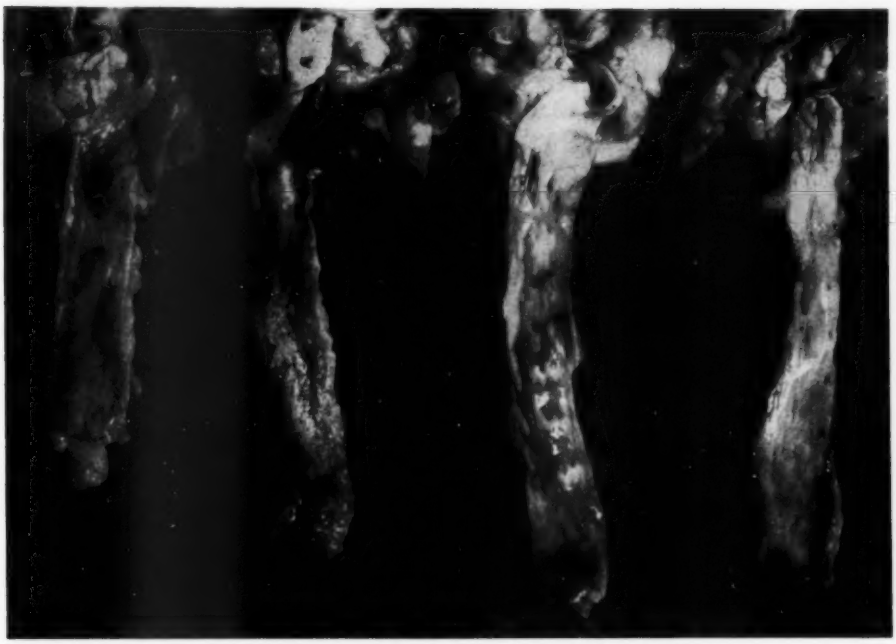


Fig. 2.—Aortas from animals given the high-cholesterol diet alone.

Fig. 3.—Aortas from animals given the high-cholesterol diet plus stearic acid.

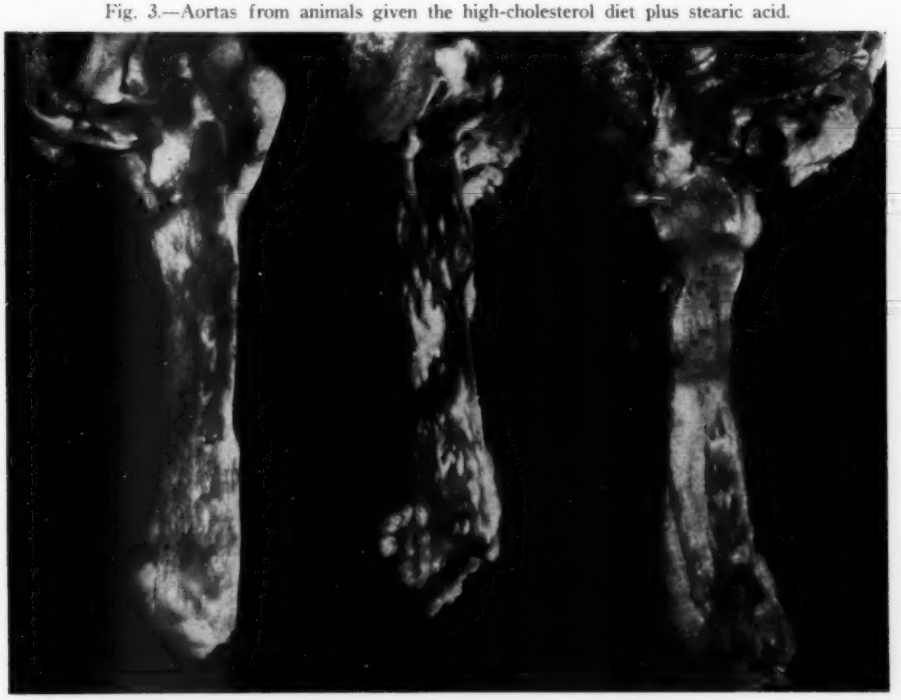


Fig. 4.—Aortas from animals given the high-cholesterol diet plus oleic acid.

Fig. 5.—Aortas from animals given the high-cholesterol diet plus linolenic acid.

The situation in experimental animals has been somewhat different. Portman, with monkeys [9]; Swell [10] and Karvinen,[11] with rats, and Chakravarti [12] and Kritchevsky,[13] with rabbits have not only shown no decrease in serum cholesterol levels after vegetable oils but in most instances have shown an increase. This is confirmed in this experiment. Kritchevsky et al. fed rabbits a high-cholesterol diet.[14] To some of these a saturated oil was added; to others, an unsaturated oil. Those to which the saturated oil was added and those to which no oil supplement was given showed demonstrably more atherosclerosis than those fed the cholesterol plus the unsaturated oil. Kritchevsky extended this work further.[13] Again using rabbits, he utilized the methyl esters of stearic, oleic, and octa 9-11 dienoic acids with a high-cholesterol diet. Once again he noted that as the unsaturated increased so did the serum cholesterol levels. However, less atherosclerosis resulted. Peifer and his group confirmed this in pigs.[15] The above experiment utilized the fatty acids themselves with similar results.

It is now clear that the effect on cholesterol metabolism which is manifested when vegetable fat is ingested is, in large part at least, due to the unsaturated fatty acids contained therein. This experiment was designed to render the rabbits hypercholesteremic and to maintain a high level of cholesterol intake while adding small amounts of fatty acids of different saturation. The mechanism behind the above-noted reactions is not as yet clear, but there are plausible hints. Lin has shown that unsaturated fats actually aid in cholesterol absorption from the intestine, so that their antiatherogenic effect is scarcely applicable at this level.[16] Similarly, the above studies have shown elevated serum cholesterol levels when unsaturated fats are added. Hepatic cholesterosis was severest in two of our linolenic acid-fed rabbits. Hirsch has done much to further a solute-solvent theory.[17] It is maintained that the cholesterol deposit in the arteries is directly proportioned to its insolubility in the serum. The solubility of the unsaturated fatty acid esters of cholesterol is thought to be greater than that of the saturated fatty acid esters. As a result, the former is kept in the circulation longer and is subjected to whatever "normal" catabolic processes are available. Presumably, these in the rabbit are few, and as a result the liver becomes packed with cholesterol. In animals better suited for cholesterol excretion this is of yet greater importance. In our experiments, we looked for differences in reaction on the two acids of increasing amounts of unsaturation. With our methods we were unable to detect any difference between those fed oleic acid and those fed linolenic acid. The linolenic acid was kept stoppered but not in vacuum, and so undoubtedly some unsaturation was lost. This may be a factor.

The experimental production of atherosclerosis in laboratory animals deficient in pyridoxine likewise projects into this area. Pyridoxine is thought to be concerned in the enzymatic processes of the unsaturated-fatty acid metabolism.

Despite several leading clinical trials, the eventual import of the unsaturated fats when applied to human atherosclerosis is not established. Keys feels that this consideration is a minor one.[18] Yet it is clear that the type of fat as well as the quantity should be considered in this disease.

Summary

Twenty rabbits were fed high-cholesterol diets, with twelve of these receiving a supplement of fatty acids with varying amounts of saturation.

Those that received the unsaturated-fatty acid supplements developed distinctly less atherosclerosis than those fed cholesterol alone or cholesterol plus a saturated fatty acid.

The unsaturation appears to have increased the serum cholesterol levels above the levels of the animals fed cholesterol

alone or those with a saturated acid addition. No alteration in serum lipid phosphorus or total serum lipids is apparent.

REFERENCES

1. Ahrens, E. H., Jr.; Blankenhorn, D. H., and Tsaltas, T. T.: Effect on Human Serum Lipids of Substituting Plant for Animal Fat in the Diet, Proc. Soc. Exper. Biol. & Med. 86:872, 1954.

2. Van Itallie, T. B.: Dietary Fats and Atherosclerosis, Nutrition Rev. 15:1, 1957.

3. Kinsell, L. W.; Michaels, G. D.; Cochrane, G. C.; Partridge, J. W.; John, J. P., and Balch, H. E.: Effect of Vegetable Fat on Hypercholesterolemia and Hyperphospholipidemia: Observations on Diabetic and Nondiabetic Subjects Given Diets High in Vegetable Fat and Protein, Diabetes 3:113, 1954.

4. Swell, L.; Flick, D. F.; Field, H., Jr., and Treadwell, C. R.: Role of Fat and Fatty Acid in Absorption of Dietary Cholesterol, Am. J. Physiol. 180:124, 1955.

5. Kinsell, L. W.: Effects of High-Fat Diets on Serum Lipids: Animal vs. Vegetable Fats, J. Am. Dietetic A. 30:685, 1954.

6. Ahrens, E. H., Jr.; Hirsch, J., Jr.; Insull, W., Jr.; Tsaltas, T. T.; Blomstrand, R., and Peterson, M. L.: The Influence of Dietary Fats on Serum-Lipid Levels in Man, Lancet 1:943, 1957.

7. Bronte-Stewart, B.; Antonis, A.; Eales, L., and Brock, J. F.: Effects of Feeding Different Fats on Serum-Cholesterol Level, Lancet 1:521, 1956.

8. Beveridge, J. M. R.; Connell, W. F.; Mayer, G. A.; Firstbrooke, J. B., and DeWolfe, M. S.: The Effects of Certain Vegetable and Animal Fats on the Plasma Lipids of Humans, J. Nutrition 56:311, 1955.

9. Portman, O. W.; Hegsted, D. M.; Stare, F. J.; Bruno, D.; Murphy, R., and Sinisterra, L.: Effect of the Level and Type of Dietary Fat on the Metabolism of Cholesterol and Beta Lipoproteins of the Cebus Monkey, J. Exper. Med. 104:817, 1956.

10. Swell, L., and Flick, D. F.: Effect of Dietary Fat and Cholesterol on the Blood Cholesterol Level in Rats, Am. J. Physiol. 174:51, 1953.

11. Karvinen, E.; Lin, T-M., and Ivy, A. C.: Effect of Certain Dietary Fats on the Absorption of Cholesterol, Am. J. Physiol. 179:650, 1954.

12. Chakravarti, R. N.; De, U. N., and Mukerji, B.: Studies on Experimental Atherosclerosis: I. Effect of Feeding Different Vegetable Fats on the Pathogenesis of Atherosclerosis in Animals (Rabbit), Indian. J. M. Res. 44:49, 1956.

13. Kritchevsky, D.; Moyer, A. W.; Tesar, W. C.; McCandless, R. F. J.; Logan, J. B.; Brown, R. A., and Englert, M. E.: Cholesterol Vehicle in Experimental Atherosclerosis: II. Influence of Unsaturation, Am. J. Physiol. 185:279, 1956.

14. Kritchevsky, D.; Moyer, A. W.; Tesar, W. C.; Logan, J. B.; Brown, R. A.; Davies, M. C., and Cox, H. R.: Effect of Cholesterol Vehicle in Experimental Atherosclerosis, Am. J. Physiol. 178:30, 1954.

15. Peifer, J. J., and Lundberg, W. O.: Influence of Specific Fatty Acids on the Development of Atherosclerosis in Miniature Pigs, Fed. Proc. 16:232, 1957.

16. Lin, T-M.; Karvinen, E., and Ivy, A. C.: Relation of Dietary Fat to the Absorption and Elimination of Exogenous and Endogenous Cholesterol, Am. J. Physiol. 183:86, 1955.

17. Hirsch, E. F., and Nailor, R.: Atherosclerosis: IV. The Relation of the Composition of the Blood Lipids to Atherosclerosis in Experimental Hyperlipemia, A. M. A. Arch. Path. 61: 469, 1956.

18. Keys, A.: Deficiency of Essential Fatty Acids, Letters to the Editor, Lancet 1:576, 1957.

The Histogenesis of Antibody-Producing Intraocular Transplants of Thymus in Mice

WILLIAM L. WILLIAMS, M.D.; WILLIAM M. HALE, M.D., Memphis, and RICHARD D. STONER, Ph.D., Upton, L. I., N. Y.

Introduction

Increasing interest is being given to the specific cells that produce antibodies. Most authors are in agreement that an antigen is accepted by cells of the reticuloendothelial system and may be demonstrated by fluorescent techniques for considerable periods after administration of the antigen in both immature and mature cells of the lymphoid series, including plasma cells but not lymphocytes.[1] The failure to demonstrate antigens in lymphocytes has led most observers to abandon this concept of origin of antibodies. Marshall[2] points out the facts in favor of and against both the plasma-cell and the germinal-center theories of origin. The available evidence leads him to suggest that the antigen is phagocytized by a metallophilic cell and subsequently transferred to a primitive reticular cell, with ensuing maturation along the plasma-cell series. We have previously emphasized the importance of the primitive reticular cell in the response to an antigen as evidenced by the ability of these undifferentiated cells to produce tetanus antitoxin in the absence of mature cells.[3]

Hale and Stoner[4,5] have demonstrated the production of tetanus antitoxin by intraocular transplants of thymus, spleen, lymph nodes, and Peyer's patches in previously irradiated recipient mice. It is our purpose to describe the histogenesis of transplants of thymus within the anterior chamber of mouse eyes and to emphasize the changes in cellular morphology which occur during the time when these transplants are capable of responding to the antigen as demonstrated by subsequent production of antitoxin. We shall illustrate that at the time the antigen is given the presence of mature cells is not essential for antibody production. Tetanus antitoxin is produced after the injection of the specific antigen, when the thymic transplants consist of immature cells of the reticuloendothelial system.

Submitted for publication Jan. 6, 1958.

This research was supported in part by the Atomic Energy Commission.

Presented in part at the American Society for Experimental Pathology, Federation of American Societies for Experimental Biology, Chicago, April, 1957.

Institute of Pathology, University of Tennessee (Drs. Williams and Hale) and Medical Department, Brookhaven National Laboratory (Dr. Stoner). Present address (Dr. Williams): Mercy Hospital, Charlotte, N. C.

Methods and Materials

Mice.—The Swiss Albino mice used in these experiments were from the same strain used in previous studies for testing antitoxin formation by intraocular transplants of the thymus. They were raised in our own colony and have been maintained as an intact strain for about 19 years. Female mice 4 to 6 weeks of age were used as recipients in all experiments.

Radiation and Transplantation.—The radiation source consisted of 21 slugs of Co^{60} arranged to give a uniform field of exposure 12 in. above the source. At the time of these experiments the intensity of the source was 425 rep per hour. Female mice 4 to 6 weeks of age were given 650 rep of whole-body γ-radiation 24 hours prior to their use as recipients. This amount of γ-radiation is normally sublethal for our mice 4 weeks of age. All animals serving as recipients in these experiments were given Co^{60} radiation. Aseptic technique was used to obtain tissues from nonirradiated donor mice that had previously been immunized with tetanus toxoid. These tissues were placed in 0.85% saline, cut into fragments about 1 mm. in diameter, and transplanted into the anterior chamber of each eye of the irradiated mice.

Histology.—The animals were killed at 12, 24, and 48 hours and 3, 4, 6, 8, 10, 15, 20, 30, 40, and

Effect of Time of Antigenic Stimulus After Intraocular Transplantation upon Ability of Thymic Transplants to Produce Tetanus Antitoxin *

Group	Mice, No.	Transplant (in Each Eye)	Toxoid Injection I. V. of Recipients, Days After Transplantation †	Fraction of I. U./Ml. of Pooled Serum	M. L. D. of Tetanus Toxin Neutralized by 1 Ml. Pooled Serum, No.
1	28	Thymus	2	0.0016	62.5
2	25	None (irradiated control)	2	<0.00004	<1.5
3	49	Thymus	4	0.0025	100.0
4	38	None (irradiated control)	4	<0.00004	<1.5
5	45	Thymus	6	0.0081	325.5
6	46	None (irradiated control)	6	<0.00004	<1.5
7	43	Thymus	10	0.00094	37.5
8	41	None (irradiated control)	10	<0.00004	<1.5
9	24	Thymus	30	0.0015	6.0
10	23	None (irradiated control)	30	<0.00004	<1.5
11	24	Thymus	60	0.00008	3.0
12	22	None (irradiated control)	60	0.00008	3.0
13	25	Thymus	No injection ‡	<0.00004	<1.5

* All animals given 650 rep whole-body γ-radiation.
† Recipient mice were irradiated 24 hours prior to intraocular transplantation. The period of time between radiation and injection of toxoid is the same for recipient groups and corresponding radiation control groups.
‡ Animals killed and serum obtained 68 days after transplantation.

60 days after transplantation. The eyes were removed and fixed in either Zenker's acetate or formaldehyde. Paraffin sections were made and stained with hematoxylin and eosin, Giemsa stain, methyl green pyronin, Wilder's silver stain, and the Weil-Davenport silver method.

Experimental Procedures and Results

A series of experiments was undertaken to test the ability of intraocular transplants of thymus to produce tetanus antitoxin at various periods of time following transplantation. The data summarized in the Table are a compilation of several experiments. Female donor mice were given a primary stimulus of 0.05 ml. of alum-precipitated tetanus toxoid (Lederle *) when 4 to 5 weeks of age. The second stimulus consisted of an intra-abdominal injection of 0.05 ml. of fluid tetanus toxoid (Lederle) given 20 to 30 days after the primary stimulus. The donor tissue was taken for transplantation 10 to 20 days after the second injection of toxoid. Donor animals varied in age from 9 to 12 weeks when they were killed for thymic tissue. All recipient animals and irradiated control groups and transplanted groups of mice were given 650 rep of whole-body γ-radiation. Both irradiated control groups and transplanted groups of mice were given an intravenous injection by tail vein of 0.2 ml. of 1:4 saline dilution of fluid tetanus toxoid (Lederle). The time of antigenic stimulus after radiation and transplantation of tissues is shown in the Table. The transplanted and irradiated control groups were killed, and pooled serum was obtained eight days after intravenous injection of fluid toxoid. A detailed description of procedures used in the toxin-antitoxin titrations may be found in a previous report.

It is evident from the data summarized in the Table that intraocular transplants of thymus produced significant amounts of tetanus antitoxin when stimulated by the specific antigen 2 to 10 days after transplantation. A small amount of antitoxin was found in the pooled serum obtained from Groups 9 and 11. The transplants in these groups were given an antigenic stimulus 30 to 60 days after transplantation.

Groups 11 and 12 had equal titers of antitoxin, indicating that the irradiated control animals (Group 12) have recovered sufficiently from 650 rep of γ-radiation so as to give a small primary antibody response to an intravenous antigenic stimulus administered 60 days after exposure to radiation. This suggests that the antitoxin found in Group 11 was produced by the recipient animals instead of being formed by transplants of thymus. Group 13 served as a control of the amount of antitoxin that was passively introduced into the recipient animals in the transplants of thymus taken

* Lederle Laboratories Division, American Cyanamid Company, Pearl River, N. Y., provided a supply of tetanus toxin and tetanus toxoid.

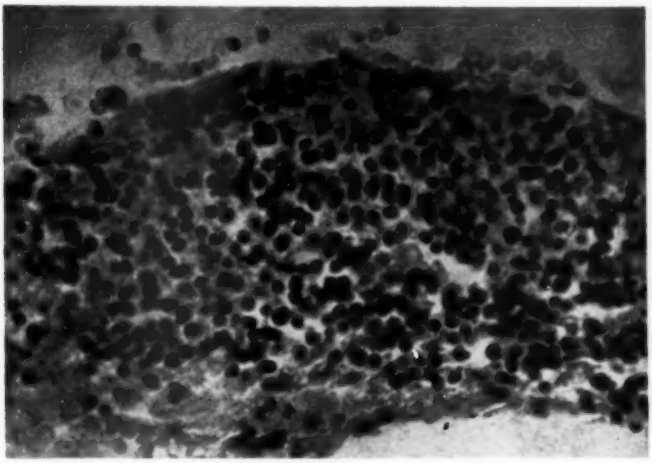


Fig. 1.—Anterior-chamber thymus transplant 12 hours after transplantation. Early necrosis. Both karyolysis and karyorrhexis are taking place. Giemsa stain; reduced about 40% from mag. × 475.

from immunized donor mice. In previous studies with similar passive control groups we have occasionally found enough antitoxin in their pooled serum to neutralize 6 to 12 M. L. D. of tetanus toxin when the serum was obtained 12 to 15 days after transplantation. Antigenic stimulation of the thymus transplants with fluid toxoid elicits a twentyfold to fiftyfold increase in antitoxin formation above the amounts found in positive control groups. In the present study a significant antibody response was demonstrated by the specific anamnestic reaction of intraocular thymus tissue when tetanus toxoid was given 2 to 10 days after transplantation.

Morphology and Transplants

The first change noted in the transplants is necrosis of the mature cells. This begins within a few hours and is well established by 12 hours, as illustrated in Figure 1. The necrosis of the mature cells progresses through 24 (Fig. 2) and 48 hours (Fig. 3). Phagocytosis of the cytoplasmic and nuclear debris is carried out by the primitive reticular metallophilic cells. By the third day necrosis is practically complete. The

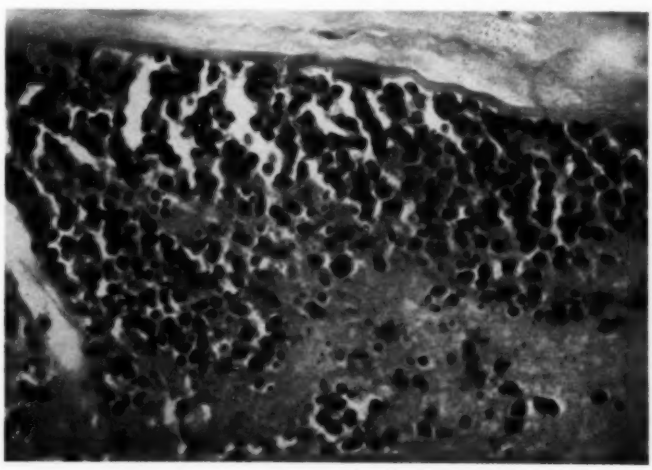


Fig. 2.—Anterior-chamber thymus transplant 24 hours after transplantation. More extensive karyorrhexis with beginning phagocytosis of nuclear debris. Hematoxylin and eosin; reduced about 40% from mag. × 475.

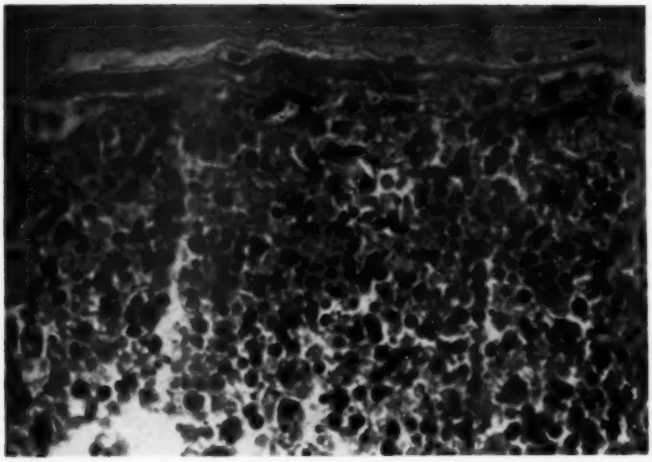


Fig. 3.—Anterior-chamber thymus transplant 48 hours after transplantation. Continuation of phagocytosis and karyorrhexis. Hematoxylin and eosin; reduced about 40% from mag. × 475.

foreign material has usually been removed, and only the more immature cells of the reticuloendothelial system remain. These cells form two basic types. One is an elongated spindle cell with short processes and little or no branching. The other is a rounded phagocytic cell (Fig. 4). Metallophilia is unpredictable at this time. By the fifth day the majority of the cells are the elongated or rod type, with occasionally the rounded forms and frequently the phagocytic cell still discernible (Fig. 5). Occasionally elongated forms and frequently the phagocytic or rounded types accept the silver stain at this stage (Fig. 6).

By 14 days the transplants consist usually of a syncytium of cells with abundant slightly eosinophilic cytoplasm containing oval, rather large, vesicular nuclei (Figs. 7 and 8). Mature lymphocytes and plasma cells have not yet evolved.

Occasionally by 20 days the transplants consist of mature cells of the lymphoid series with surrounding scar tissue and even fat (Figs. 9 and 10). The more usual picture even as long as 60 days post-transplantation consists of a background of syncytial and elongated primitive reticuloendothelial cells with scattered transitional forms and mature lymphocytes and plasma

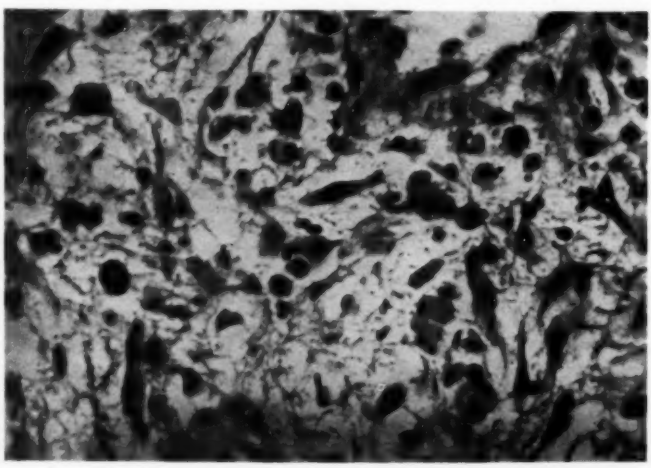


Fig. 4.—Anterior-chamber thymus transplants three days after transplantation. Nuclear debris practically removed. Note marked stromal edema and the elongated and round-cell types remaining. Hematoxylin and eosin; reduced about 40% from mag. × 475.

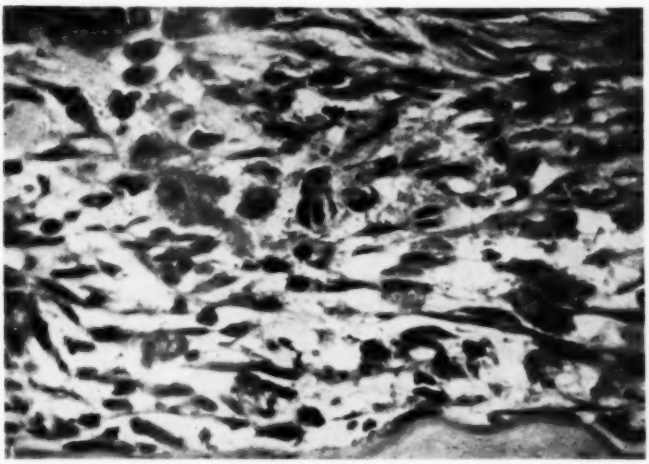


Fig. 5.—Anterior-chamber thymus transplant five days after transplantation. Only round and elongated cell types present. Transplants stimulated at this stage have been shown to produce antitoxin. Hematoxylin and eosin; reduced about 40% from mag. × 475.

cells (Fig. 11). Some of the round and rod-shaped forms are metallophilic; however, still present in the background are the large nuclei of cells with varying amounts of cytoplasm which do not stain with silver salts (Fig. 12).

Comment

Any discussion of the cells which make up organs such as thymus, lymph nodes, spleen, and bone marrow is clouded by virtue of confusing terminology and the lack of consistent methods for the identification of various cell types. This difficulty was pointed out by Aschoff in his original description of the reticuloendothelial system and has not been improved since. Aschoff considered the reticuloendothelial system to be made up of cells which were concerned in the formation of the reticulum of the lymphoid organs or which lined blood and lymph sinuses. The inclusion or exclusion of cells in the reticuloendothelial system rested primarily on the basis of the response of the cell to vital dyes or particulate materials. Recently Marshall has suggested the term reticular tissue, which he defines as "a tissue composed of fixed

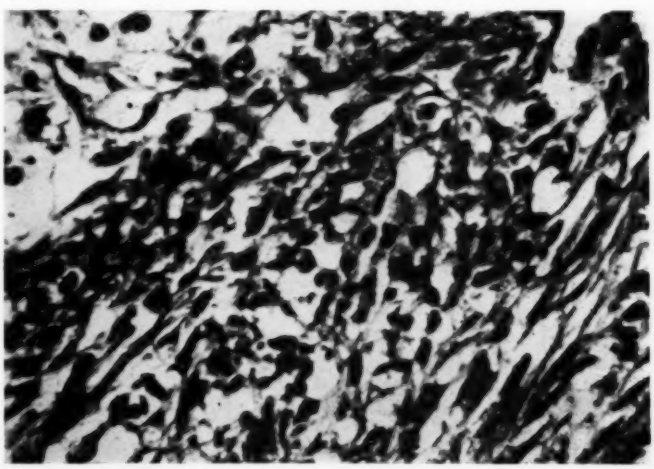


Fig. 6.—Anterior-chamber thymus transplants five days after transplantation. Same animal as Figure 5. Some rod type. Some round forms accept the silver stain at this stage. Silver impregnation; reduced about 40% from mag. × 475.

Fig. 7.—Anterior-chamber thymus transplant 14 days after transplantation. A syncytium of immature reticuloendothelial cells without mature lymphocytes or plasma cells. Hematoxylin and eosin; reduced about 30% from mag. × 430.

cells (primitive reticular cells) supported on a framework of fibrils and including all cells in the body derived from this tissue."

Cells which are present in a structure such as the thymus include lymphocytes and, at least occasionally, plasma cells with their progenitors. There is also a group of cells with large vesicular nuclei. These cells cannot be separated morphologically; however, some are and some are not phagocytic. The mature cells can be identified in both tissue sections and smears by their morphologic characteristics. The parent cells are somewhat in dispute. Most authors feel that there is a primitive cell of common origin. This primitive cell can-

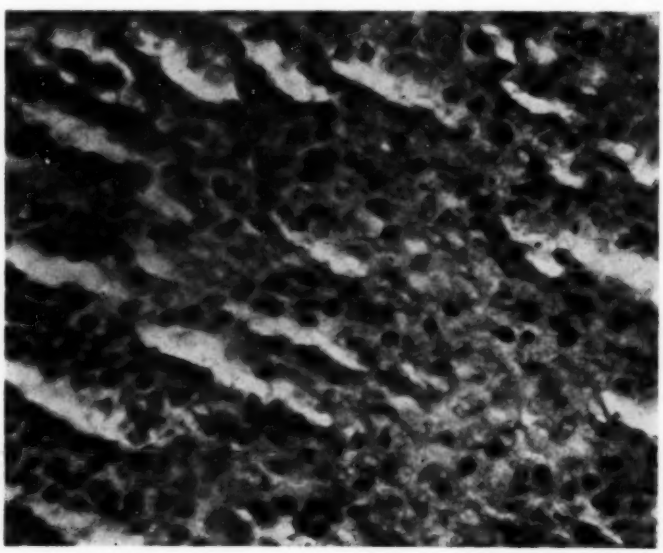


Fig. 8.—Anterior-chamber thymus transplant 14 days after transplantation. Higher magnification of Figure 7, reemphasizing the absence of mature cells. Hematoxylin and eosin; reduced about 30% from mag. × 475.

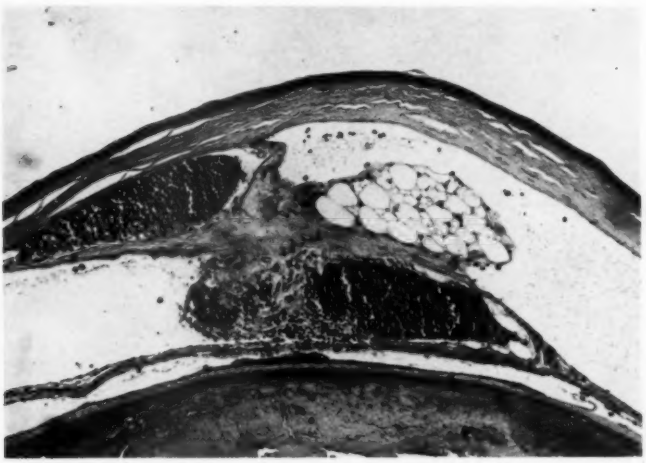


Fig. 9.—Anterior-chamber thymus transplant 20 days after transplantation. Almost lobule formation, with fat regeneration and mature lymphocytes present. Hematoxylin and eosin; reduced about 40% from mag. × 100.

not be identified with certainty in each case. The phagocytic cells are demonstrable by the use of vital stains and by their phagocytic properties in reacting to particulate materials. These phagocytic cells can also be identified by their reaction to metallic stains. It is believed that all of the cells which are phagocytic will accept the silver stain, though all metallophilic cells are not phagocytic. When one has identified these immature types of cells by means of either phagocytosis or metallophilia, there still remains another group of fixed cells which are closely associated with the reticular framework. These latter cells have been termed the primitive reticular cells or undifferentiated mesenchymal cells. They are morphologically inseparable from the cells which will accept the silver stain and those which may be phagocytic that are closely fixed to the reticulum.

At the time at which transplants of thymus were tested for antitoxin production the transplants contained no mature cells. The cell types which are demonstrable fall into three groups. The first is a rounded metallophilic cell. It is present by the third day and gradually disappears

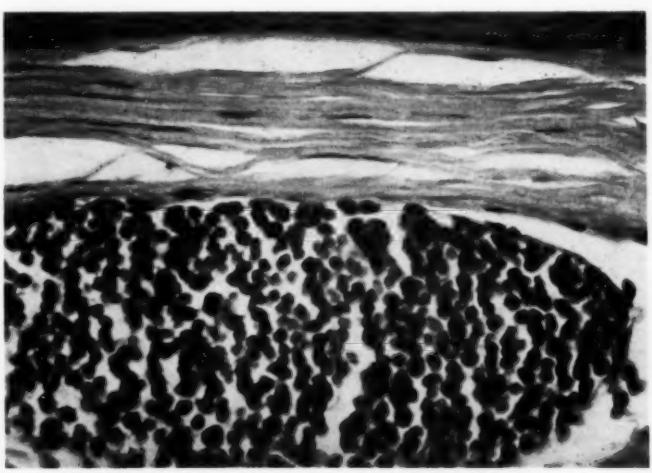


Fig. 10.—Anterior-chamber thymus transplant 20 days after transplantation. Same animal as Figure 9. Illustrates presence of mature cell forms. Hematoxylin and eosin; reduced about 40% from mag. × 475.

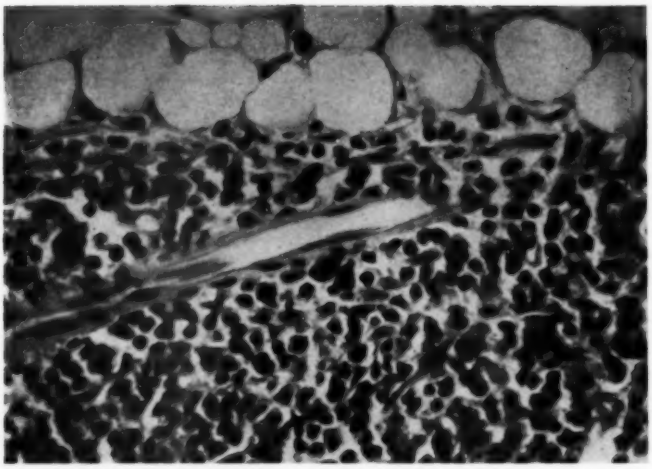


Fig. 11.—Anterior-chamber thymus transplants 60 days after transplantation. Background of syncytial elongated and rounded cell types with scattered transitional and mature forms. Hematoxylin and eosin; reduced about 40% from mag. × 475.

as the transplant resumes the usual structure of the thymus, which may be as early as the 20th day. The second cell is a rod-shaped metallophilic cell which early has few or no processes. These cells as they elongate resemble very closely fibroblasts, though no collagen is usually formed. These cells are present as early as the rounded form but remain in the mature transplant as a stellate cell. The processes develop as the transplant matures. The third cell type present has an almost identical nucleus which is vesicular without prominent nucleoli. The cytoplasm is usually indistinct and cannot be identified with metallic impregnations. These cells in the latter stages of maturation assume a syncytial pattern. These fall into the group of primitive reticular cells. The transplants of thymus within one to two months resume the lobular pattern of the organ from which they were taken.

It is our conclusion that mature lymphocytes and plasma cells are not essential for a response to an antigen. This has been demonstrated by the production of antitoxin by transplants at periods when no mature cells are present. These findings do not preclude the possible production of antibody

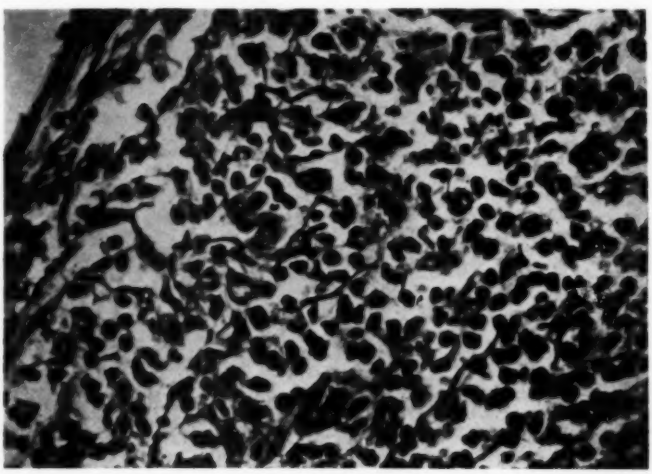


Fig. 12.—Anterior-chamber thymus transplants 60 days after transplantation. Rod-shaped and round forms accept the silver stain. Scattered in the background are many immature reticuloendothelial cells which are not metallophilic. Silver impregnation; reduced about 40% from mag. × 475.

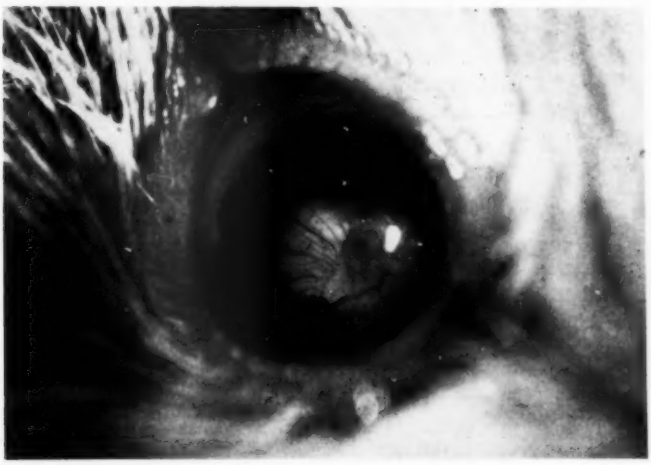


Fig. 13.—An intraocular transplant of thymus tissue 15 days after transplantation; reduced about 40% from mag. × 17.

by mature cells. Our findings indicate that it is the immature cells which are present in these transplants that have the ability to respond to the antigen and produce tetanus antitoxin. Of the three cell types present we cannot assess a difference between them of their activity in the role of antibody production.

Summary

The fate of transplants of thymus within the anterior chambers of the eyes of irradiated mice is described. The morphology of the transplants is correlated with their ability to respond to a secondary stimulus or tetanus toxoid with the production of antitoxin.

The transplant at the time the antigen is given is shown to consist of three cell types: (1) a rounded metallophilic cell, (2) a rod-shaped metallophilic cell, (3) a primitive nonmetallophilic cell. All or one of these cell types is capable of responding to tetanus toxoid, as evidence by the production of antitoxin.

Institute of Pathology, University of Tennessee, 858 Madison Ave. (3).

REFERENCES

1. Coons, A. H.; Leduc, E. H., and Connolly, J. M.: Studies on Antibody Production: I. A Method for the Histochemical Demonstration of Specific Antibody and Its Application to a Study of the Hyperimmune Rabbit, J. Exper. Med. 102: 49-60, 1955.

2. Marshall, A. H. E.: An Outline of the Cytology and Pathology of the Reticular Tissue, Springfield, Ill., Charles C Thomas, Publisher, 1956, p. 106.

3. Williams, W. L.; Stoner, R. D., and Hale, W. M.: A Correlation of Early Radiation Changes in Lymphatic Tissues with Antitoxin-Producing Ability, Yale J. Biol. & Med. 28:615-621, 1956.

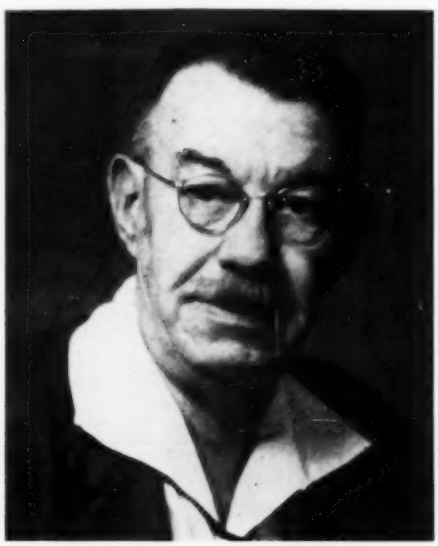
4. Hale, W. M., and Stoner, R. D.: Formation of Tetanus Antitoxin by Spleen and Lymph Node Intraocular Transplants, Yale J. Biol. & Med. 26: 46-54, 1953.

5. Stoner, R. D., and Hale, W. M.: Antibody Production by Thymus and Peyer's Patches Intraocular Transplants, J. Immunol. 75:203-208, 1955.

Obituaries

ERIC A. FENNEL, M.D.
1887-1957

Dr. Eric A. Fennel, a co-founder of the Straub Clinic at Honolulu, died on Dec. 24, 1957. He was the son of a physician and was born in Cincinnati, on Sept. 24, 1887. His grandfather had been a founder of the Cincinnati College of Pharmacy. He graduated at the Ohio State University and received his Doctor of Medicine degree at the University of Cincinnati in 1912. After his internship at the Jewish Hospital in Cincinnati, he joned the Department of Health, Cincinnati, and became Medical Director of the City Infirmary and later joined the faculty of the City College of Dentistry. He later attended the Army Medical School, and after his graduation, in October, 1917, became an instructor at the Army Medical School from 1917 to 1919. As a major in World War I he helped develop triple typhoid and lipopneumococcus vaccines and extended his



ERIC A. FENNEL, M.D.
1887-1957

experiments with the use of vaccines on cholera and dysentery. He came to Hawaii just after World War I with the Army and was in charge of the Tripler Laboratory. In 1920 he resigned from the Armed Services and was one of the founders of the Straub Clinic at Hawaii, where he helped establish the hospital laboratory at Queen's Hospital and was pathologist to the Queen's, Children's, St. Francis, and other hospitals on the islands. He organized training programs for medical technologists at the University of Hawaii and the Society of Medical Technologists.

After obtaining a leave of absence from his work as pathologist in the various institutions in Hawaii, he was appointed associate professor of pathology at the University of Illinois College of Medicine, 1928-1929. It was at this time that he became acquainted with the late Prof. A. Maximow, whose studies on tissue cultures stimulated Fennel to apply these methods in his experimental work. As a teacher he stimulated his students in the fields of science, literature, and the arts, and his clinical pathologic conferences were well attended and were one of the most stimulating exercises in his teaching program. For many years Dr. Fennel conducted research along experimental and clinical lines on leprosy, and he was responsible for some of the progress made in the diagnostic and therapeutic management of this disease. His work on leprosy in Hawaii was one of the important contributions in this field.

He established the first blood bank in Hawaii and the first plasma bank in Honolulu in 1939.

Dr. Fennel was a member of many scientific societies, including the American Society of Clinical Pathologists, and he was counselor for Hawaii, the College of American Pathologists, Certified American Board of Pathology, The American Medical Association, and Alpha Omega Alpha. He was a highly cultured man, with interests in music and the arts, and was a staunch supporter of the Honolulu Symphony Orchestra and the Academy of Arts. His flair for writing attracted men of letters, and among his colleagues was the late Henry L. Mencken, who was so impressed with Dr. Fennel's literary talents that he saved all his correspondence and later donated this to the Public Library of New York City.

Dr. Fennel has contributed more than fifty original articles in various scientific publications and more than sixty-five articles in the proceedings of the clinic, published in Hawaii.

He was endowed with an indefatigable energy and was consistently and scrupulously honest in his methods and endeavor and absolutely true to his friends and colleagues. His attitude toward his fellow men was one of kindness to the point of self-sacrifice, of liberality in judgement, and of willingness to be of help and assistance whenever needed. He was not afraid of confession of ignorance or driving home a point of conviction by logic and wit and was always true to himself and the matter under consideration.

In 1913 he married Nancey Beirne Nickell, of West Virginia, and is survived by his widow, a son and daughter, and three granddaughters.

A pathologist, scientist, and man of letters of the type of Eric Fennel contributed to the heritage of his students, colleagues, and friends, and his self-sacrifice and contributions to mankind will be an everlasting guide post in our way of life.

S. A. Levinson, M.D.

ALFRED SABATO GIORDANO, M.D.

1893-1958

Alfred S. Giordano died in his sleep at his winter home in Sarasota, Fla., on Feb. 15.

With his passing an era of American clinical pathology came to an end. Dr. Giordano was one of those few who were responsible for the development of clinical pathology as an independent division of medicine. Looked at askance, especially by traditional anatomic pathologists, the early clinical pathologists had to fight for recognition of their specialty as a respectable branch of medical science and medical practice. Dr. Giordano was in the forefront of this battle

ALFRED SABATO GIORDANO, M.D.
1893-1958

almost from the very beginning. When illness forced him to retire, he had the good fortune to see the battle won and clinical pathology not only recognized but highly esteemed by the entire medical profession.

Dr. Giordano was born in Avellino, Italy, Feb. 3, 1893. He came to the United States at the age of fourteen. The two handicaps of being an immigrant and not knowing the language of the land did not stop him from completing high school in Syracuse, N. Y., then college and medical school at Syracuse University.

He received the degrees of Bachelor of Science in 1918 and Doctor of Medicine in 1920. He chose pathology as his life's work and the Mayo Clinic as the place to learn it. He spent three years there and in 1923 received the degree of Master of Science in Pathology from the University of Minnesota. In addition to a thorough education in pathology and clinical pathology, Dr. Giordano collaborated with Dr. F. C. Mann on a fundamental study of the sphincter of the choledochus, which has been quoted widely by students of the subject.

From the very start the young pathologist had a clear idea of what he wanted. He wanted to practice bedside pathology. To him clinical pathology meant the application of pathology to the bedside problems of clinical medicine. He heard of an opportunity to do just that in South Bend, Ind. There ten years earlier a group of twenty-three physicians decided that they could not serve their patients without a good laboratory, and they founded the South Bend Medical Laboratory. In 1923 Dr. Giordano became the head of the laboratory. Under his leadership it became a model institution, unique in its service to an entire community and beyond.

In August, 1923, Dr. Giordano married Dr. Alice Gracy.

From a modest beginning the South Bend Medical Laboratory increased gradually in size, facilities, and service and later became a nonprofit organization, the South Bend Medical Foundation. It serves the private physicians, several hospitals in South Bend and neighboring communities, and the City and County Health Laboratories.

Dr. Giordano had a highly developed sense of professional responsibility. He served faithfully as Secretary-Treasurer of the American Society of Clinical Pathologists from 1930 to 1948. These were formative years, and Dr. Giordano has contributed more than anybody else to the growth of the Society. He had a hand in every important development in clinical pathology during the many years of his association with the American Society of Clinical Pathologists. These included the Annual Tumor Seminars, the Registry of Medical Technologists, the Board of Pathology (Member of the Board of Trustees), the College of American Pathologists, and the *American Journal of Clinical Pathology* (Member of the Editorial Board). In recognition of his services, Dr. Giordano received the Certificate of Highest Merit and the Ward Burdick Medal, the two highest awards the American Society of Clinical Pathologists can bestow on its distinguished members. In an editorial in the *American Journal of Clinical Pathology* (19:51, 1949), entitled "Dr. Giordano, Our True Physician," he was given credit as having "contributed more to the advancement of Clinical Pathology than any other individual."

Dr. Giordano understood the importance of participation in local, state, and national activities of organized medicine. He was past president of the St. Joseph County Medical Society, a delegate to the Indiana State Medical Association and to the American Medical Association, and chairman of the advisory board for the study of blood banks of the American Medical Association. He was chairman of a medical board of the South Bend-St. Joseph County Veteran Service Commission and was instrumental in the establishment of a tumor registry of the St. Joseph County Cancer Society. He was prominently active in the Community Chest, Red Cross, and Y. M. C. A. He loved music and was actively supporting the South Bend symphony.

Dr. Giordano had a genuine interest in medical research. He made significant contributions to the epidemiology of brucellosis and wrote five frequently quoted papers on the subject which were responsible for a South Bend ordnance requiring pasteurization of milk.

His paper "Occurrence of a non-specific substance in guinea pig serum fixed by antigen in the Wassermann test" (*Am. J. Clin. Path.* 9:130-135 [March] 1939) attracted wide attention, as did one of his hematologic contributions, "Idiopathic hypoprothrombinemia" (*Am. J. Clin. Path.* 13:285-287 [June] 1943). His thirty-nine papers dealt with serology of syphilis, hematology, blood grouping, blood chemistry, parasitology, endocrinology, bacteriology, pathologic anatomy, and problems of methodology. They reveal the extent of his scientific interests.

He is survived by his wife and four children (Dr. Robert Giordano, New York City; Mrs. Carol McMillan, Rochester, N. Y.; Mrs. Nancy Gobar, Pasadena, Calif., and Lt. David Giordano, a medical officer in the Navy), six grandchildren, a brother, and sister.

Dr. Giordano was a man of deep convictions and of great courage. He knew how to compromise when necessary, but he never compromised when basic principles were involved. He was a good and devoted friend of every clinical pathologist and was actively interested in our problems even when illness confined him to his home. Jerry's last letter to me was dated Jan. 4, 1958. In it he wrote: "Even though I have been relegated to the background. . . . I am still very much alive as to the broad interests of Pathology. I am particularly happy to note the recent elections into prominent place in both College and the A. S. C. P. and I hope that the A. S. C. P. will see fit to find a qualified layman to assume the burden of the Secretary office routine."

He was able to view his illness in a detached manner. This is how he expressed it in the same letter: "As to my health I am about the same except with less cardiac reserve as evidenced by the more frequent night attacks of left ventricular failure particularly when my salt intake is increased. That of course prevents me from traveling and I have given up the idea of visiting Italy."

Jerry will be long remembered as the grand man of American Clinical Pathology.

I. Davidsohn, M.D.

News and Comment

ANNOUNCEMENTS

Meeting of American Public Health Association.—The American Public Health Association holds its annual meeting in St. Louis, Oct. 27-31, 1958. Further information can be obtained from the American Public Health Association, 1790 Broadway, New York 19.

Research on Connective Tissue Diseases.—The Helen Hay Whitney Foundation has announced the availability of research fellowships to provide "adequate early financial support for young postdoctoral men and women interested in careers in clinical and nonclinical investigation dealing with connective tissue and its diseases." The foundation was established in 1947 to stimulate and support research in rheumatic fever and rheumatic heart disease and has recently expanded its activities to include the study of connective tissue and its diseases. Any qualified person, wherever located, up to the age of 35, holding the M.D., Ph.D., or their equivalent, is eligible. The applicant must have a commitment for adequate facilities and research expenses during the tenure of the fellowship from an institution or laboratory acceptable to the Scientific Advisory Committee. The fellowship is an annual award to continue for a period of three years contingent on performance satisfactory to the advisory committee. Stipends will be individualized to meet the needs of the fellow. A contribution of $1000 will be made to the laboratory of the fellow selected. For applications and information write the Helen Hay Whitney Foundation, 525 E. 68th St., Room F-231, New York 21. Applications should be mailed prior to Aug. 15 for fellowships commencing July 1 of the following year.

Award Science Fellowships.—The National Science Foundation has announced the award of 54 Science Faculty Fellowships for the academic year 1958-1959. These fellowships are offered "as a means of improving the teaching of science, mathematics, and engineering in American colleges and universities." The primary purpose of the awards is to provide an opportunity for college and university science teachers to enhance their effectiveness as teachers. The foundation also announced award of 27 Senior Postdoctoral Fellowships in the sciences for the academic year 1958-1959, selected from 155 applicants. Ten awards were made in the life sciences, and fifteen, in the physical sciences, including a number in interdisciplinary fields. Applications were reviewed by a panel of scientists under arrangements made by the National Research Council. Science Faculty and Senior Postdoctoral Fellowships carry stipends adjusted to approximate the regular salaries of award recipients. These stipends may be applied toward study or research in an accredited nonprofit institution of higher learning in the United States or abroad. All awards were approved by the National Science Board upon the recommendation of Alan T. Waterman, Ph.D., director of the foundation.

SOCIETY NEWS

Rhode Island Medical Society.—Dr. Shields Warren of Boston addressed the annual meeting of the Rhode Island Medical Society on May 14, 1958. His title was "The Prevention of Somatic and Genetic Radiation Injury."

New Officers for American Society of Clinical Pathologists.—The following officers have been installed for the American Society of Clinical Pathologists: President, Dr. Harry P. Smith, New York; President-Elect, Dr. Edward L. Burns, Toledo, Ohio, and Vice-President, Dr. Joe M. Blumberg, Washington, D. C.

DEATHS

Dr. Francisco Duran-Reynals.—Dr. Francisco Duran-Reynals, of Yale University School of Medicine, died on March 27, 1958, at the age of 58. Dr. Duran-Reynals will be remembered for his discovery of hyaluronidase and for his pioneering investigations of viruses in relation to cancer.

PERSONAL

Inauguration of Dr. Robert A. Moore.—Dr. Robert A. Moore was formally inaugurated as the first president of the State University of New York College of Medicine, Downstate Medical Center, on May 2, 1958.

Dr. Jethro Gough Gives Lecture.—Dr. Jethro Gough, professor of pathology in the Welsh National School of Medicine at Cardiff, Wales, gave a lecture at the Mayo Clinic and Mayo Foundation, Rochester, Minn., on April 21, 1958. Dr. Gough spoke on "Some Recent Investigations in Pulmonary Pathology."

Dr. Lewis Thomas Gives Lecture.—Dr. Lewis Thomas, Chairman, Department of Pathology, New York University College of Medicine, gave the sixth annual Emanuel B. Schoenbach Memorial Lecture in the Maimonides Hospital of Brooklyn on April 24, 1958. His title was "Role of Hypersensitivity in Tissue Damage and Disease."

Dr. John Higginson Joins University of Kansas Medical Center.—Dr. John Higginson, formerly with the South African Institute for Medical Research and Baragwanath Hospital, Johannesburg, South Africa, has joined the Department of Pathology and Oncology at the University of Kansas Medical Center as Associate Professor.

Award to Dr. Kenneth M. Lynch.—Dr. Kenneth M. Lynch, President of the Medical College of South Carolina, has received the Southern Medical Association's Distinguished Service Award. Dr. Lynch has been Professor of Pathology at the Medical College of South Carolina since 1913. He was made dean of the College in 1944 and president in 1949.
